CG000153 Rev A

USER GUIDE

Chromium Single Cell DNA Reagent Kits

FOR USE WITH

Chromium Single Cell DNA Library & Gel Bead Kit, 16 rxns PN-1000040
Chromium Single Cell DNA Cell Bead Kit, 16 rxns PN-1000056
Chromium Chip C Single Cell DNA Kit, 48 rxns PN-1000022
Chromium Chip D Single Cell DNA Kit, 48 rxns PN-1000042
Chromium i7 Multiplex Kit, 96 rxns PN-120262

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Notices

Document Number

CG000153 | Rev A

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 Genomics 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 Genomics Limited Warranty, Assurance Plan or service agreement will not be replaced or repaired.

Chromium Controller Usable Temperature Range

The recommended temperature range for the Chromium Controller or the Chromium Single Cell Controller when running the protocol outlined in this Chromium Single Cell DNA Reagents Kits User Guide is 19-25°C (66-77°F), which is different than the usable temperature range of 18-28°C (64-82°F) stated in the Chromium Controller Specifications (Document CG00020) and the Chromium Single Cell Controller Specifications (Document CG00050). Running the Chromium Controller or the Chromium Single Cell Controller outside the recommended temperature range of 19-25°C (66-77°F) when using the reagent kits and chip kits described herein will invalidate the warranty of these products.

Support

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Chromium Single Cell DNA Reagent Kits

Chromium Single Cell DNA Library & Gel Bead Kit, 16 rxns PN-1000040

Chromium Single Cell DNA Library Kit, 16 rxns PN-1000025 (store at –20°C)

Chromium
Single Cell DNA
Reagents Module 1

	#	PN
Single Cell DNA Enzyme Mix	1	2000031
Single Cell DNA Reagent Mix	1	2000032
Additive A	1	220074
Buffer Sample Clean Up 1	1	220020

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Chromium
Single Cell DNA
Reagents Module 2

	#	PN
End Repair and A-tailing Buffer	1	220120
End Repair and A-tailing Enzyme	1	220121
Ligation Buffer	1	220109
DNA Ligase	1	220110
Adaptor Mix	1	220026
Amplification Master Mix	1	220125
Forward PCR Primer	1	220124

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Chromium Single Cell DNA Gel Bead Kit, 16 rxns PN-1000026 (store at –80°C)

Chromium
Single Cell DNA
Gel Beads

	#	PN
Single Cell DNA Gel Beads	2	2000033

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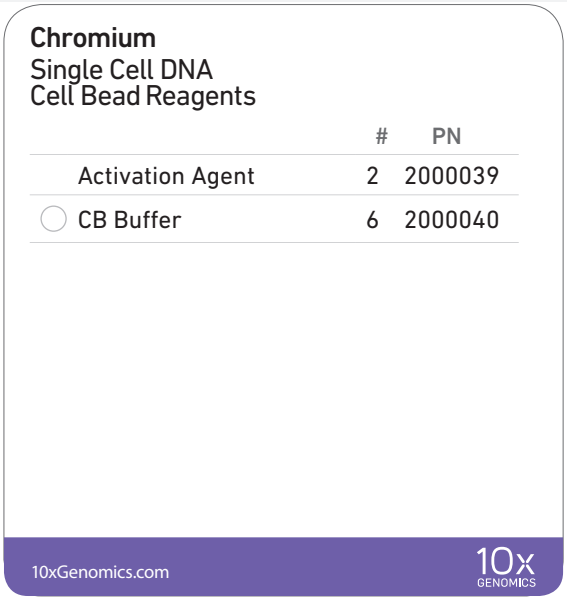
Dynabeads™ MyOne™ SILANE, 16 rxns PN-2000048 (store at 4°C)

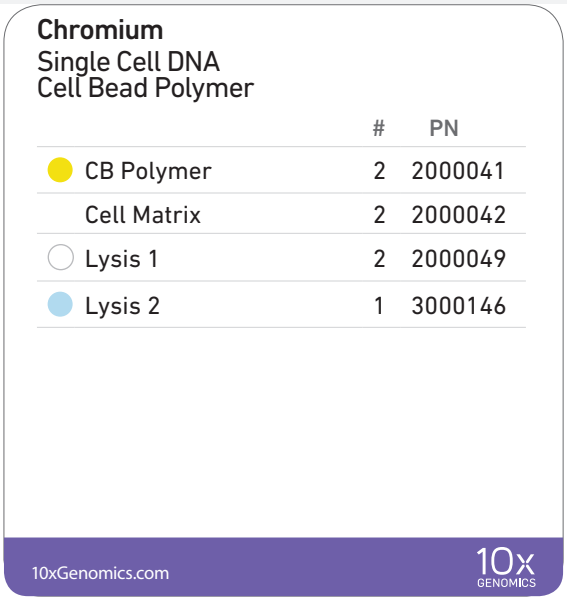
	#	PN
Dynabeads MyOne SILANE	2	2000048

Chromium Single Cell DNA Cell Bead Kit, 16 rxns PN-1000056

Chromium Single Cell DNA Cell Bead Reagent Kit, 16 rxns PN-1000023 (store at –20°C)



Chromium Single Cell DNA Cell Bead Polymer Kit, 16 rxns PN-1000031 (store at 4°C)



Chromium Chip C Single Cell DNA Kit, 48 rxns PN-1000022 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	6	220088

Chromium
Recovery Agent

	#	PN
Recovery Agent	6	220016

Chromium
Chip C & Gaskets

	#	PN
Chip C Single Cell DNA	6	2000030
Gasket, 6-pack	1	370017

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Chromium Chip D Single Cell DNA Kit, 24 rxns PN-1000042 (includes PN-1000021, 2 kits) (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	6	220088

Chromium
Recovery Agent

	#	PN
Recovery Agent	6	220016

Chromium
Chip D & Gaskets

	#	PN
Chip D Single Cell DNA	6	2000037
Gasket, 6-pack	1	370017

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Chromium i7 Multiplex Kit, 96 rxns PN-120262 (store at –20°C)

Chromium
i7 Multiplex Kit

	#	PN
Chromium i7 Sample Index Plate	1	220103

Flowmi™ Filters, 50 rxns, PN-1000055

	#	PN
Flowmi	1	1000055

Chromium Single Cell DNA Accessory Kit, PN-1000058

Product	#	PN (Orderable)	PN (Item)
10x Magnetic Separator A	1	1000054*	2000067
10x Chromium Chip D Holder	1	1000053*	3000109
CABLE USB A MALE - B MALE 1M BLK	1	-	3000173

*May be ordered individually or as part of the Chromium Single Cell DNA Accessory Kit. Also available as part of the Chromium Controller Accessory Kit, PN-110204.

Chromium Accessories

Product	PN (Orderable)	PN (Item)
10x Vortex Adapter	120251	330002
10x Vortex Clip	120253	230002
10x Chip Holder	120252	330019
10x Magnetic Separator	120250	230003

Recommended Thermal Cyclers

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

Supplier	Description	Part Number
BioRad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	1851197
Eppendorf	MasterCycler Pro	North America 950030010 International 6321 000.01
Thermo Fisher Scientific	Veriti 96-Well Thermal Cycler	4375786

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Chromium Single Cell DNA protocol. Substituting materials may adversely affect system performance.

Supplier	Description	Part Number (US)
Plastics		
Eppendorf	PCR Tubes 0.2 ml 8-tube strips DNA LoBind Tubes, 1.5 ml DNA LoBind Tubes, 2.0 ml	951010022 022431021 022431048
USA Scientific	TempAssure PCR 8-tube strip	1402-4700
Rainin	Tips LTS W-O 200UL Filter RT-L200WFLR Tips LTS 20UL Filter RT-L10FLR Tips LTS 200UL Filter RT-L200FLR Tips LTS 1ML Filter RT-L1000FLR	30389241 30389226 30389240 30389213
Kits & Reagents		
Thermo Fisher Scientific	Nuclease-free Water	AM9937
Corning Cellgro	Phosphate-Buffered Saline (PBS) 1X without calcium and magnesium	21-040-CV
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous)	E7023-500ML
	1 N NaOH	S2770-100ML
	Phosphate-Buffered Saline (PBS) with 10% Bovine Albumin	SRE0036
Beckman Coulter	SPRIselect Reagent Kit	B23318
Bio-Rad	10% Tween 20	1610781
Ricca Chemical Company	Glycerin (glycerol), 50% (v/v) Aqueous Solution	3290-32
Teknova	1 M Tris-HCl, pH 7.2	T1072
Qiagen	Qiagen Buffer EB	19086
Equipment		
VWR	Vortex Mixer Divided Polystyrene Reservoirs	10153-838 41428-958
Eppendorf	Eppendorf ThermoMixer C Eppendorf SmartBlock PCR 96 Eppendorf SmartBlock 1.5 ml, Thermoblock for 24 Reaction Vessels	5382000023 5306000006 5360000038
Invitrogen	DynaMag-2 Magnet (holds 1.5-ml microcentrifuge tubes)	12321D

Choose either Eppendorf or USA Scientific PCR 8-tube strips.

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Chromium Single Cell DNA protocol. Substituting materials may adversely affect system performance.

Supplier	Description		Part Number (US)
Equipment			
Rainin	Pipet-Lite LTS Pipette L-2XLS Pipet-Lite LTS Pipette L-10XLS Pipet-Lite LTS Pipette L-20XLS Pipet-Lite LTS Pipette L-100XLS Pipet-Lite LTS Pipette L-200XLS Pipet-Lite LTS Pipette L-1000XLS Pipet-Lite Multi Pipette L8-10XLS Pipet-Lite Multi Pipette L8-20XLS Pipet-Lite Multi Pipette L8-50XLS Pipet-Lite Multi Pipette L8-200XLS		17014393 17014388 17014392 17014384 17014391 17014382 17013802 17013803 17013804 17013805
Quantification & Quality Control			
Agilent	2100 Bioanalyzer Laptop Bundle High Sensitivity DNA Kit Agilent DNA 1000 Kit 4200 TapeStation High Sensitivity D1000 ScreenTape High Sensitivity D1000 Reagents	Choose Bioanalyzer or TapeStation, based on availability and preference.	G2943CA 5067-4626 5067-1504 G2991AA 5067-5592 5067-5593
KAPA Biosystems	KAPA Library Quantification Kit for Illumina Platforms		KK4824

Protocol Steps & Timing

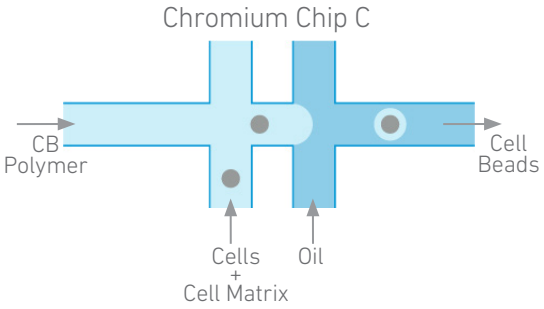
Day	Steps		Timing	Stop & Store
Day 1 2 h	**Cell Preparation**			
	Dependent on Cell Type		~1 h	
	Step 1 – Generate Cell Beads			
	1.1	Load Chromium Chip C	40 min	
	1.2	Run the Chromium Controller & Transfer Cell Beads	20 min	Incubate on a thermomixer overnight
Day 2 4 h	**Step 2 – Process Cell Beads**			
	2.1	Solvent Exchange	20 min	
	2.2	Lysis	1 h 30 min	
	2.3	Filtration	20 min	STOP –80°C ≤3 months
	Step 3 – GEM Generation & Barcoding			
	3.1	Prepare Reaction Mix	10 min	
	3.2	Load Chromium Chip D	15 min	
	3.3	Run the Chromium Controller	5 min	
	3.4	Transfer GEMs	10 min	
	3.5	GEM Isothermal Incubation	8 h 10 min	STOP 4°C ≤72 h or –20°C ≤1 week
Day 3 6 h	**Step 4 – Post GEM Incubation Cleanup & QC**			
	4.1	Post GEM Incubation Cleanup – Dynabeads	40 min	
	4.2	Post GEM Incubation Cleanup – SPRIselect	20 min	STOP 4°C ≤72 h or –20°C ≤2 weeks
	4.3	Post GEM QC	50 min	
	Step 5 – Library Construction			
	5.1	End Repair & A-tailing	1 h 15 min	
	5.2	Adaptor Ligation	20 min	
	5.3	Post Ligation Cleanup – SPRIselect	20 min	
	5.4	Sample Index PCR	40 min	STOP 4°C ≤72 h
	5.5	Post Sample Index Double Sided Size Selection – SPRIselect	20 min 25 min	STOP 4°C ≤72 h or –20°C long-term
	5.7	Post Library Construction QC	50 min	
	5.8	Post Library Construction Quantification	20 min	

Stepwise Objectives

The Chromium Single Cell CNV Solution provides a comprehensive, scalable approach to determine genomic heterogeneity and map clonal evolution by profiling hundreds to thousands of cells in a single sample. This is achieved by encapsulating individual cells in a hydrogel matrix to generate Cell Beads on a microfluidic chip. The Cell Bead is treated to lyse the encapsulated cell and denature the genomic DNA (gDNA). On a second microfluidic chip, GemCode Technology samples a pool of ~750,000 10x Barcodes to separately index the gDNA of each individual cell. It does so by partitioning Cell Beads into nanoliter-scale Gel Beads-in-emulsion (GEMs), where all fragments share a common 10x Barcode. Libraries are generated and sequenced and 10x Barcodes are used to associate individual reads back to the individual partitions, and thereby, to each individual cell.

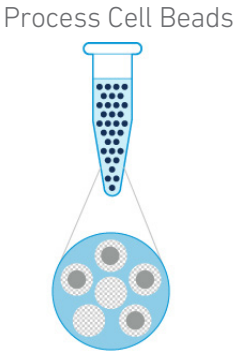
Step 1 Cell Bead Generation

Cell Beads are generated by partitioning individual cells or nuclei in a hydrogel matrix by combining Cell Matrix with CB Polymer on a Chromium Chip C. Cells are delivered at a dilution such that the majority of generated Cell Beads contain either a single cell or are empty, with minimal multiplets. Cell Beads contain magnetic particles for ease of downstream handling. The Cell Beads are incubated on a thermomixer overnight.



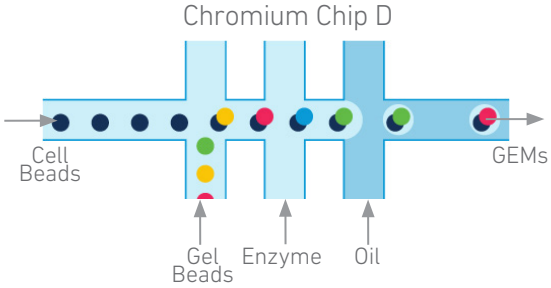
Step 2 Cell Bead Processing

Cell Beads are recovered after removing the Partitioning Oil. Enzymatic and chemical treatment of Cell Beads lyses the encapsulated cell and denatures the gDNA. The denatured gDNA in the Cell Bead is accessible for amplification and barcoding. The Cell Bead suspension is filtered.

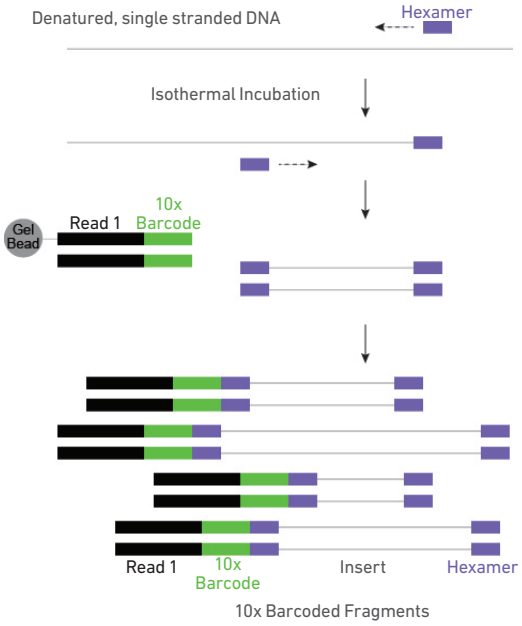


Step 3 GEM Generation & Barcoding

GEMs are generated by combining barcoded Gel Beads, Cell Beads, a Master Mix, and Partitioning Oil on a Chromium Chip D. To achieve single cell resolution, a single Cell Bead and a single Gel Bead are co-encapsulated in majority (~80%) of generated GEMs.



Immediately after GEM generation, the Gel Bead and Cell Bead are dissolved. Oligonucleotides containing (i) an Illumina R1 sequence (Read 1 primer sequence) and (ii) a 16 nt 10x Barcode are released and mixed with hexamers and the denatured gDNA. Amplification and barcoding of the gDNA during two-step isothermal incubation of the GEMs produces 10x barcoded fragments ranging from tens to thousands of base pairs. After incubation, the GEMs are broken and pooled fractions are recovered.

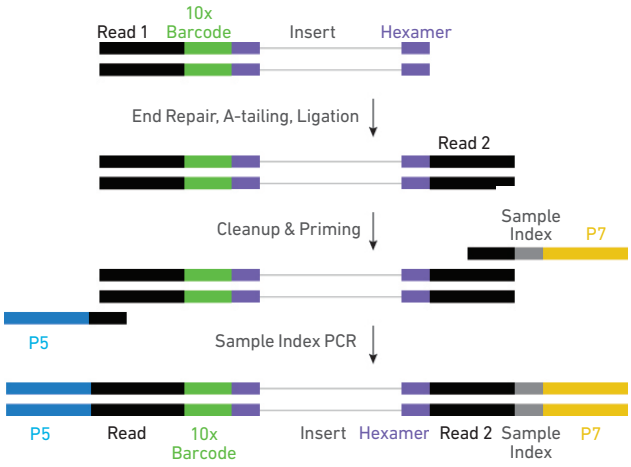


Step 4 Post GEM Incubation Cleanup & QC

Silane magnetic beads are used to remove leftover biochemical reagents from the post GEM reaction mixture. Solid Phase Reversible Immobilization (SPRI) beads are used to optimize the DNA size range for library construction.

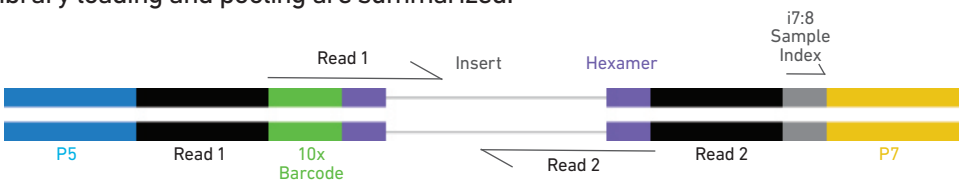
Step 5 Library Construction

P5, P7, a sample index, and an Illumina R2 sequence (Read 2 primer sequence) are added via End Repair, A-tailing, Adaptor Ligation, and PCR. The final libraries contain the P5 and P7 priming sites used in Illumina sequencers.



Sequencing

The Chromium Single Cell DNA protocol produces Illumina-ready sequencing libraries. Illumina sequencer compatibility, sample indices, sequencing depth & run parameters, library loading and pooling are summarized.



Tips & Best Practices

Icons

Tip & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

Emulsion-safe Plastics

- Use 10x Genomics validated emulsion-safe plastic consumables when handling GEMs as some plastics can destabilize GEMs.

Multiplet Rate

Multiplet Rate (%)	# of Cells Loaded	# of Cells Recovered
~0.2%	~1,600	~250
~0.4%	~3,100	~500
~0.7%	~6,300	~1,000
~1.4%	~12,500	~2,000
~2.1%	~18,800	~3,000
~2.9%	~25,000	~4,000
~3.6%	~31,300	~5,000

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 rxn values.
- Cover Partitioning Oil tubes and reservoirs to minimize evaporation.
- 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:

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

  ii. Filter through a 0.2-µm filter.

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

Pipette Calibration

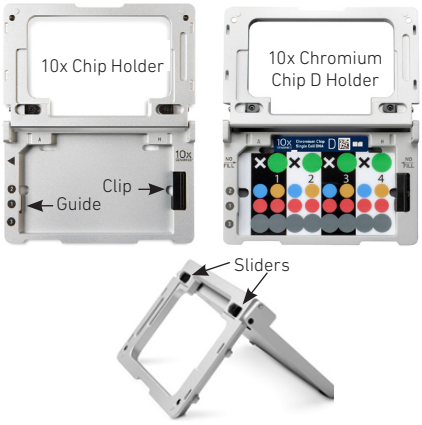
- Follow manufacturer's calibration and maintenance schedules.
- Pipette accuracy is **critical** in **Step 2** and also, when using SPRIselect reagents.

Chromium Chip Handling

- Minimize exposure of reagents, chips, and gaskets to sources of particles and fibers, laboratory wipes, frequently opened flip-cap tubes, and dusty surfaces.
- Execute steps without pause or delay, unless indicated. When multiple chips are to be used, load, run, and collect the content from one chip before loading the next.
- Fill all unused input wells in rows labeled 1, 2, and 3 on a chip with an appropriate volume of 50% glycerol solution before loading the used wells. DO NOT add glycerol to the Recovery Wells.
- Avoid contacting the bottom surface of the chip with gloved hands and other surfaces. Frictional charging can lead to inadequate priming of the channels, potentially leading to either clogs or wetting failures.
- Minimize the distance that a loaded chip is moved to reach the Chromium Controller.
- Keep the chip horizontal to prevent wetting the gasket with oil, which depletes the input volume and may adversely affect the quality of the assay.

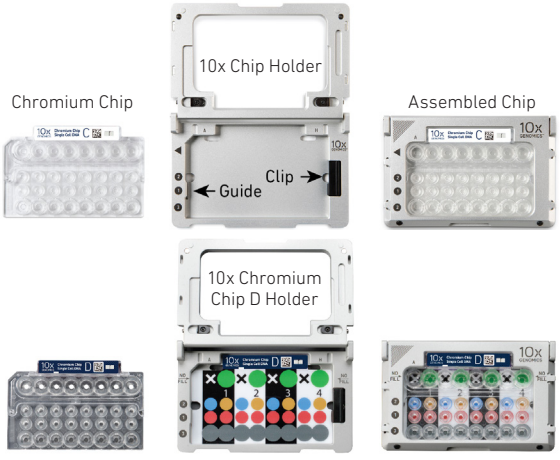
10x Chip Holders

- 10x Chip Holders encase Chromium Chips.
- The holder lid flips over to become a stand, holding the chip at 45 degrees for optimal Recovery Well content removal.
- Squeeze the black sliders on the back side of the holder together to unlock the lid and return the holder to a flat position.
- Use the 10x Chip Holder to encase Chip C.
- Use the 10x Chromium Chip D Holder (includes a color-coded well map) to encase Chip D.



Chromium Chip & Holder Assembly

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



Activation Agent Handling

- Use one tube of Activation Agent per sample. DO NOT puncture foil seal of tubes not used at the time.
- Store unused tubes at –20°C.

Cell Matrix Handling

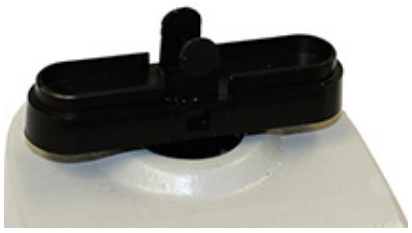
- Use one tube of Cell Matrix per sample. DO NOT puncture foil seal of tubes not used at the time.
- Store unused tubes at 4°C. DO NOT freeze Cell Matrix.

Chromium Chip C Loading

- Place the assembled chip and holder flat on the bench with the lid closed.
- Dispense at the bottom of the wells without introducing bubbles.
- Wait for the Cell Bead Mix to drain into the bottom of the pipette tips and dispense again to ensure complete volume transfer.
- Refer to Load Chromium Chip C 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.
- Equilibrate the Gel Beads strip to room temperature before use.
- Store unused Gel Beads at –80°C and avoid more than 10 freeze-thaw cycles. DO NOT store Gel Beads at –20°C.



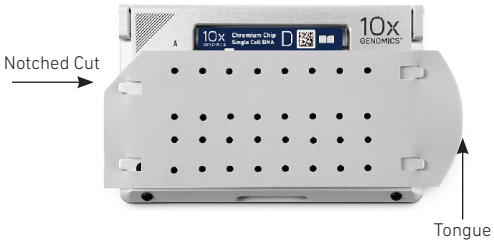
- Attach a 10x Vortex Adapter to the top of standard laboratory vortexers to vortex the Gel Bead strips.
- After vortexing, remove the Gel Bead strip from the adapter. Flick the Gel Bead strip in a sharp, downward motion to maximize Gel Bead recovery. Confirm there are no bubbles at the bottom of the tubes.
- 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.

Chromium Chip D Loading

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

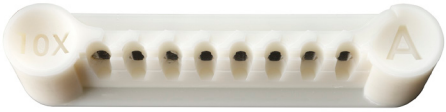
10x Gasket Attachment

- After reagents are loaded, attach the gasket by holding the tongue (curved end, to the right), aligning the notch with the top left-hand corner, and then hooking 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. DO NOT press down on the top of the gasket after attachment.
- Keep the assembly horizontal to avoid wetting the gasket with Partitioning Oil.



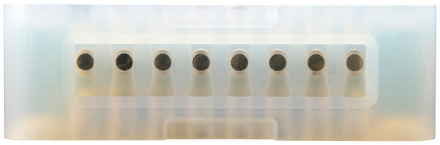
10x Magnetic Separator A

- Offers one position of the magnets relative to a tube.
- 10x Magnetic Separator **A** is different from 10x Magnetic Separator.
- Use for Cell Bead Processing.



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 or low positions.
- DO NOT use for Cell Bead Processing.



SPRIselect Cleanup & Size Selection

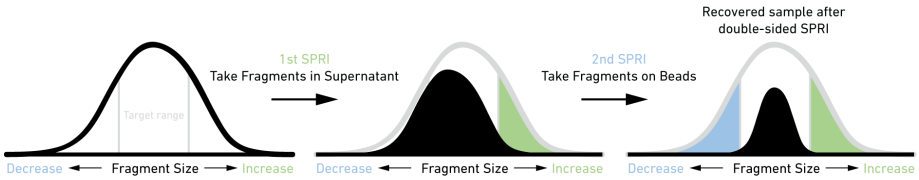
- After aspirating the desired volume of SPRIselect reagent, examine the pipette tips before dispensing to ensure the correct volume is transferred.
- Pipette mix thoroughly as insufficient mixing of sample and SPRIselect reagent will lead to inconsistent results.
- Use fresh preparations of 80% Ethanol.

Tutorial — SPRIselect Reagent:DNA Sample Ratios

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

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

Schematic of Double Sided Size Selection



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

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 i7 Sample Index plate contains a unique mix of 4 oligos.
- The sample indexes can therefore be used in any combination.
- Each sample index set is base-balanced to avoid monochromatic signal issues when it is the sole sample loaded on an Illumina sequencer.

Step 1

Cell Bead Generation

1.1 Load Chromium Chip C

1.2 Run the Chromium Controller & Transfer Cell Beads

1.0
Cell Bead Generation

GET STARTED!				
Action	**Item**	**10x PN**	**Preparation & Handling**	**Storage**
Equilibrate to Room Temperature	**1X PBS**	-	-	-
Place on Ice	**Activation Agent** (Cell Bead Reagent Kit)	2000039	Use one tube per sample. Puncture the tube foil seal immediately before use. Return unused tubes to –20°C. 	–20°C
	Cell Matrix (Cell Bead Polymer Kit)	2000042	Use one tube per sample. Puncture the tube foil seal immediately before use. Return unused tubes to 4°C. 	4°C
	CB Polymer (Cell Bead Polymer Kit)	2000041	-	4°C
	Cells/Nuclei in 1X PBS + 0.04% BSA	-	DO NOT use >0.04% BSA as it may inhibit Cell Bead formation.	-
Obtain	**Partitioning Oil**	220088	-	Ambient
	Nuclease-free Water	-	-	-
	Chromium Chip C	2000030	See Tips & Best Practices.	Ambient
	10x Vortex Adapter	330002	See Tips & Best Practices.	Ambient
	10x Gasket	370017	See Tips & Best Practices.	Ambient
	10x Chip Holder	330019	See Tips & Best Practices.	Ambient
	50% glycerol solution If using <8 reactions	-	See Tips & Best Practices.	Ambient

Firmware Version 3.0 or higher is required in the Chromium Controller or the Single Cell Chromium Controller used for the Single Cell DNA protocol.

Cell Suspension Volume Calculator Table

Volume of 1X PBS Per Reaction (µl) | Volume of Cell Suspension Per Reaction (µl)

Cell Stock Concentration (Cells/µl)	Targeted Cell Recovery (Cells)							
	250	500	1000	2000	2500	3000	4000	5000
200	2.7 7.8	n/a	n/a	n/a	n/a	n/a	n/a	n/a
400	6.6 3.9	2.7 7.8	n/a	n/a	n/a	n/a	n/a	n/a
600	7.9 2.6	5.3 5.2	n/a	n/a	n/a	n/a	n/a	n/a
800	8.5 2.0	6.6 3.9	2.7 7.8	n/a	n/a	n/a	n/a	n/a
1000	8.9 1.6	7.4 3.1	4.2 6.3	n/a	n/a	n/a	n/a	n/a
1200	9.2 1.3	7.9 2.6	5.3 5.2	n/a	n/a	n/a	n/a	n/a
1400	9.4 1.1	8.3 2.2	6.0 4.5	1.6 8.9	n/a	n/a	n/a	n/a
1600	9.5 1.0	8.5 2.0	6.6 3.9	2.7 7.8	n/a	n/a	n/a	n/a
1800	n/a	8.8 1.7	7.0 3.5	3.6 6.9	1.8 8.7	n/a	n/a	n/a
2000	n/a	8.9 1.6	7.4 3.1	4.2 6.3	2.7 7.8	1.1 9.4	n/a	n/a
2200	n/a	9.1 1.4	7.7 2.8	4.8 5.7	3.4 7.1	2.0 8.5	n/a	n/a
2400	n/a	9.2 1.3	7.9 2.6	5.3 5.2	4.0 6.5	2.7 7.8	n/a	n/a
2600	n/a	9.3 1.2	8.1 2.4	5.7 4.8	4.5 6.0	3.3 7.2	n/a	n/a
2800	n/a	9.4 1.1	8.3 2.2	6.0 4.5	4.9 5.6	3.8 6.7	1.6 8.9	n/a
3000	n/a	9.5 1.0	8.4 2.1	6.3 4.2	5.3 5.2	4.2 6.3	2.2 8.3	n/a
3200	n/a	n/a	8.5 2.0	6.6 3.9	5.6 4.9	4.6 5.9	2.7 7.8	n/a
3400	n/a	n/a	8.7 1.8	6.8 3.7	5.9 4.6	5.0 5.5	3.1 7.4	1.3 9.2
3600	n/a	n/a	8.8 1.7	7.0 3.5	6.2 4.3	5.3 5.2	3.6 6.9	1.8 8.7
3800	n/a	n/a	8.9 1.6	7.2 3.3	6.4 4.1	5.6 4.9	3.9 6.6	2.3 8.2
4000	n/a	n/a	8.9 1.6	7.4 3.1	6.6 3.9	5.8 4,7	4.2 6.3	2.7 7.8

Grey boxes: Volumes that would exceed the allowable 1X PBS volume in each reaction
Yellow boxes: Indicate a low cell suspension transfer volume that may result in higher cell load variability

1.1 Load Chromium Chip C

See Tips & Best Practices for chip handling instructions. 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.

TIPS

a. Assemble Chromium Chip C in a 10x Chip Holder See Tips & Best Practices

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

i. **75 µl** into unused wells in **row labeled 1**.

ii. **40 µl** into unused wells in **row labeled 2**.

iii. **200 µl** into unused wells in **row labeled 3**.

DO NOT add 50% glycerol solution to the top row of Recovery Wells.
DO NOT use any substitute for 50% glycerol solution.

c. Prepare Cell Bead Mix

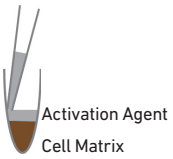
i. Remove the Activation Agent and Cell Matrix tube strips from the holders. Centrifuge briefly. Place on ice.

ii. Using a pipette tip, puncture the foil seal of one Activation Agent and one Cell Matrix tube per sample and enlarge the holes, avoiding contact between the pipette tip and the Activation Agent or the Cell Matrix.

iii. Add **20 µl** nuclease-free water to each Activation Agent tube. Pipette mix to dissolve.

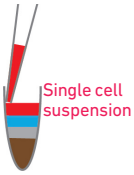
DO NOT mix during steps iv-vi.

iv. Layer **4.7 µl** Activation Agent solution on top of the Cell Matrix avoiding contact between the Cell Matrix and the pipette tip. DO NOT mix.



v. Refer to the Cell Suspension Volume Calculator Table and layer the appropriate volume of PBS on top of the Activation Agent. DO NOT mix.

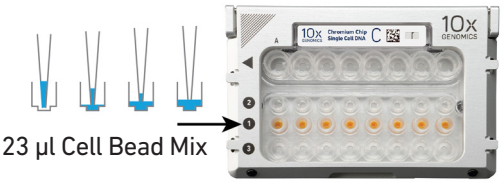
vi. Resuspend cells and **immediately** layer the corresponding volume of single cell suspension on top of PBS. DO NOT mix.



See Tips & Best Practices for chip handling instructions. 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.

d. Load Row Labeled 1

Using a **50-µl multi-channel pipette** (set to 20 µl), gently pipette mix the Cell Bead Mix until homogeneous. Using the same pipette tips, dispense **23 µl** Cell Bead Mix into the bottom center of each well in the **row labeled 1** without introducing bubbles.
Wait for the Cell Bead Mix to drain into the bottom of the pipette tips and dispense again to ensure complete transfer.



Dislodge any Cell Bead Mix stuck along the sidewalls of the well using a pipette tip. Accurate input volume is critical for correct Cell Bead volume yield and optimal performance.

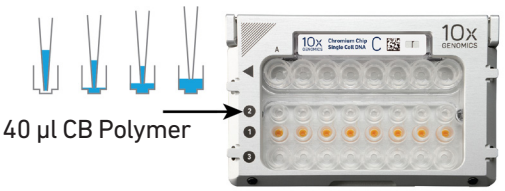
e. Prepare CB Polymer

Centrifuge the CB Polymer briefly. Vortex **10 sec**. Centrifuge briefly again.

f. Load Row Labeled 2

Using a **single-channel** pipette, slowly dispense **40 µl** CB Polymer into the bottom center of each well in the **row labeled 2** without introducing bubbles.

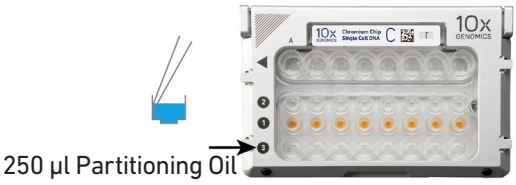
Wait **60 sec** for the chip to prime.



g. Load Row Labeled 3

Dispense **250 µl** Partitioning Oil into each well in the **row labeled 3.**

Failure to add Partitioning Oil can damage the Chromium Controller.



h. Attach 10x Gasket

Align the notch with the top left-hand corner. Ensure the gasket holes are aligned with the wells. Avoid touching the smooth gasket surface. DO NOT press down on the gasket.

1.2 Run the Chromium Controller & Transfer Cell Beads

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

b. Place the loaded chip with the gasket in the tray. Press the button to retract the tray.

c. Confirm program on screen. Press the play button.

d. At completion of the run **(~11.5 min)**, the Controller will chime. **Immediately** proceed to the next step. Steps e – i should be executed within **5 min**.

e. Press the eject button to remove the chip.

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

g. Remove **60 µl** Partitioning Oil from lowest point of the Recovery Wells in the top row using **regular-bore** pipette tips.

h. Using **wide-bore** pipette tips (pipette set to 95 µl), over the course of **~30 sec** aspirate remaining Partitioning Oil and Cell Beads from the bottom of each Recovery Well and transfer into a PCR 8-tube strip. Dispense slowly with the pipette tips against the sidewalls without introducing bubbles. Using the same pipette tips, transfer any remaining Partitioning Oil and Cell Beads from the Recovery Wells to the tubes.

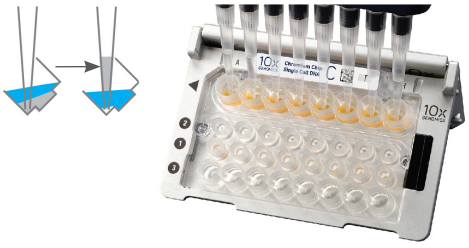
DO NOT flick, shake or vortex the tubes to prevent wetting the sidewalls and caps of the tubes.

i. **Immediately** place the capped tube strip on a thermomixer and shake at **21°C** for **16-24 h** at **1000 rpm.**

Usable Temperature Range
19-25°C (66-77°F)

Expose Wells

Remove Partitioning Oil



Transfer Cell Beads
(use wide-bore pipette tips)

Cell Beads (wide-bore pipette tips)

Step 2

Cell Bead Processing

2.1 Solvent Exchange

2.2 Lysis

2.3 Filtration

2.0 Cell Bead Processing

GET STARTED!				
Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	**1X PBS**	-	-	-
	CB Buffer (Cell Bead Reagent Kit)	2000040	-	–20°C
Place on Ice	**Lysis 1** (Cell Bead Polymer Kit)	2000049	Mix by inverting tube 5x. DO NOT vortex.	4°C
	Lysis 2 (Cell Bead Polymer Kit)	3000146	Vortex. Centrifuge briefly.	4°C
Obtain	**Recovery Agent**	220016	-	Ambient
	0.4 N NaOH Prepare 1 ml for 8 reactions	-	Prepare fresh.	Ambient
	1 M Tris-HCl, pH 7.2	-	-	Ambient
	10x Magnetic Separator A	2000067	Different from the 10x Magnetic Separator.	Ambient
	10x Vortex Clip	230002	-	Ambient
	1.5 ml magnetic tube holder	-	-	Ambient
	Flowmi Filters, 70 µm	1000055	-	Ambient

2.1 Solvent Exchange

a. Remove the tube strip containing Cell Beads from the thermomixer.

b. Remove **45 µl** Partitioning Oil from the bottom of the tube. **5-10 µl** Partitioning Oil should remain. DO NOT aspirate Cell Beads. If Cell Beads are aspirated, return the solution to the tube, reduce removal volume by **5 µl**, and reattempt removal.

c. Add **95 µl** PBS.

d. Cap the tube strip and place in a 10x Vortex Clip. Vortex **20 sec**. Centrifuge **~1 sec** only. The Cell Bead suspension may not be uniform.

e. Add **60 µl** Recovery Agent. Wait **60 sec**.

f. Gently invert ~25x. DO NOT vortex.

g. Centrifuge **10 sec** to separate the phases. The top aqueous layer contains Cell Beads. The bottom pink layer contains Recovery Agent/Partitioning Oil. Place on a 10x Magnetic Separator **A** for **30 sec.**

TIPS

10x Magnetic Separator **A** (magnet **A**) is different from 10x Magnetic Separator.

h. Insert pipette tips along the sidewalls of the tube, away from the Cell Beads and slowly remove **120 µl** Recovery Agent/ Partitioning Oil/PBS from the bottom of the tube, retaining the Cell Beads. If the level of samples remaining is not at the etched mark on magnet **A**, adjust by either adding or removing PBS.

A small volume of Recovery Agent/ Partitioning Oil may remain in the tube. DO NOT aspirate Cell Beads.

i. Add **120 µl** PBS. Remove from magnet **A**.

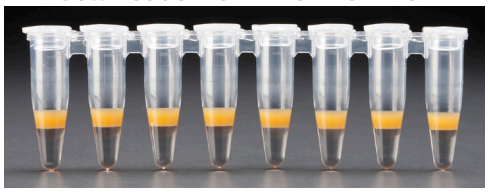
j. Gently invert until homogeneous and centrifuge **~1 sec**.

k. Place on magnet **A** for **30 sec**.

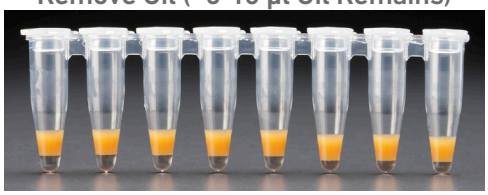
l. Remove **120 µl** from the bottom of the tube, retaining the Cell Beads.

m. Remove from magnet **A**.

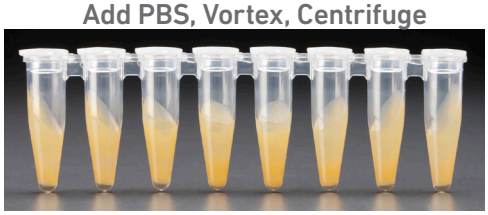
Cell Beads from Thermomixer



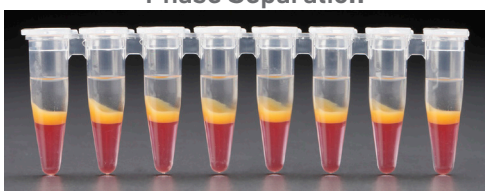
Remove Oil (~5-10 µl Oil Remains)



Add PBS, Vortex, Centrifuge

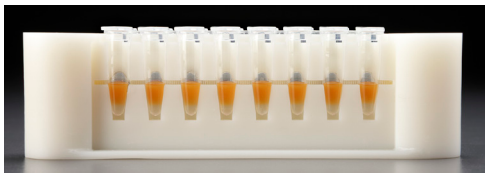


Phase Separation



Place on Magnetic Separator A

Retain Cell Beads



2.2 Lysis

a. Add **120 µl** Lysis 1. Mix by inverting until homogeneous. Centrifuge **~1 sec**.

b. Place on magnet **A** for **30 sec**.

c. Remove **120 µl** from the bottom of the tube, retaining the Cell Beads.

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

Lid Temperature	Reaction Volume	Run Time
85°C	75 µl	10 min
Step	**Temperature**	**Time**
Incubate	72°C	00:10:00
Hold	4°C	Hold

e. Add **100 µl** Lysis 1. Mix by inverting until homogeneous. Centrifuge **~1 sec**.

f. Add **5 µl** Lysis 2. **Immediately** mix by inverting until homogeneous.

g. Incubate **15 min** at **room temperature**. Gently invert every **5 min** to prevent settling of beads.

h. Centrifuge **~1 sec**. Place on magnet **A.** Wait **30 sec**.

i. Remove **105 µl** from the bottom of the tube, retaining the Cell Beads.

j. Add **120 µl** PBS. Mix by inverting until homogeneous. Centrifuge **~1 sec**.

k. Place on magnet **A.** Wait **30 sec**.

l. Remove **120 µl** from the bottom of the tube, retaining the Cell Beads.

m. Repeat steps j – l for a total of 2 washes.

n. Adjust the liquid level such that the meniscus is at the etched mark on magnet **A** by either adding or removing PBS.

o. Remove from magnet **A.** Add **75 µl** freshly prepared 0.4 N NaOH. Mix by inverting until homogeneous.

p. Incubate **5 min** at **room temperature.** Gently invert every **~1 min.** Centrifuge **~1 sec** at the end of incubation.

q. Add **50 µl** 1 M Tris-HCl, pH 7.2. Mix by inverting until homogeneous. Centrifuge **~1 sec**.

r. Place on magnet **A.** Wait **30 sec**.

s. Remove **120 µl** from the bottom of the tube, retaining the Cell Beads.

t. Add **120 µl** CB Buffer. Mix by inverting until homogeneous. Centrifuge **~1 sec**.

u. Place on magnet **A**. Wait **30 sec**.

v. Remove **120 µl** from the bottom of the tube, retaining the Cell Beads.

w. Repeat steps t – v for a total of 2 washes.

2.3 Filtration

a. Add **450 µl** CB Buffer to a 1.5-ml microcentrifuge tube for each sample.

b. Using a **single-channel** pipette (set to 75 µl), pipette mix Cell Beads. Using the same pipette tip, transfer **all** Cell Beads to the microcentrifuge tube containing CB Buffer.

c. Using a **single-channel** pipette (set to 520 µl), gently pipette mix the Cell Bead suspension (volume may be less than **520 µl**). Using the same pipette tip, aspirate the Cell Bead suspension and attach the pipette tip to a **70 µm** Flowmi filter.

Attach Flowmi Filter

d. **Immediately** pass the Cell Bead suspension slowly through the Flowmi filter to the **first stop**, into a new 1.5-ml microcentrifuge tube. Variation in volume of filtrate is normal.

Pass through Flowmi Filter

e. Place the capped tube containing the Cell Bead suspension on a 1.5-ml magnetic tube holder for **3 min** or until the solution clears.

f. Remove **260 µl** solution, retaining the Cell Beads.

g. Remove from the magnet. Pipette mix using a **200-µl single-channel pipette** (set to 185 µl) until homogeneous without introducing bubbles. Using the same pipette tip, transfer exactly **185 µl** to a new tube strip. Some Cell Beads may remain in the 1.5-ml microcentrifuge tube. If volume is less than **185 µl,** add CB buffer from the CB Buffer bottle to bring volume to **185 µl**.

Remove 159 µl

h. Centrifuge **~1 sec**. Place on magnet **A** for **3 min**.

i. Using a **200-µl single-channel pipette**, slowly remove **159 µl** from the bottom of the tube. A variable small volume of Cell Beads may be aspirated.

j. Using a **single-channel pipette,** add **7 µl** CB Buffer from the CB Buffer bottle to the retained Cell Beads. Remove from the magnet and place on ice.

STOP

k. Store at **–80°C** for up to **3 months** or proceed to the next step.

Step 3

GEM Generation & Barcoding

3.1 Prepare Reaction Mix

3.2 Load Chromium Chip D

3.3 Run the Chromium Controller

3.4 Transfer GEMs

3.5 GEM Isothermal Incubation

3.0 GEM Generation & Barcoding

GET STARTED!				
Action	**Item**	**10x PN**	**Preparation & Handling**	**Storage**
Equilibrate to Room Temperature	**Chromium Single Cell DNA Gel Beads**	2000033	Equilibrate to room temperature 30 min before loading the chip.	–80°C
	Cell Beads If stored at –80°C	-	Equilibrate to room temperature 30 min before loading the chip, centrifuge briefly.	–80°C
	Additive A	220074	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
	Single Cell DNA Reagent Mix	2000032	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
Place on Ice	**Single Cell DNA Enzyme Mix**	2000031	Maintain on ice. Store at –20°C immediately after use.	–20°C
Obtain	**Partitioning Oil**	220088	-	Ambient
	Chromium Chip D	2000037	See Tips & Best Practices.	Ambient
	10x Gasket	370017	See Tips & Best Practices.	Ambient
	10x Chromium Chip D Holder	3000109	See Tips & Best Practices.	Ambient
	10x Vortex Adapter	330002	See Tips & Best Practices.	Ambient
	50% glycerol solution If using <4 reactions	-	See Tips & Best Practices.	-

3.1 Prepare Reaction Mix

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

Reaction Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Single Cell DNA Reagent Mix	2000032	65.0	286.0	572.0
Additive A	220074	2.5	11.0	22.0
Single Cell DNA Enzyme Mix	2000031	12.0	52.8	105.6
Total	-	**79.5**	**349.8**	**699.6**

3.2 Load Chromium Chip D

See Tips & Best Practices for chip handling instructions. 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.

TIPS

a. Assemble Chromium Chip D in a 10x Chip D Holder See Tips & Best Practices

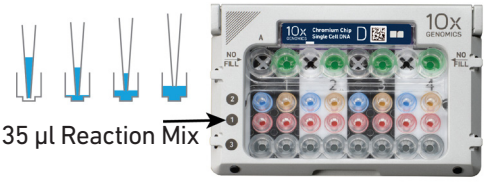
b. Dispense 50% Glycerol Solution into Unused Chip Wells (if <4 samples per chip)

i. **35 µl** into unused wells in **row labeled 1**.
ii. **40 µl** into unused wells in **row labeled 2**.
iii. **150 µl** into unused wells in **row labeled 3**.

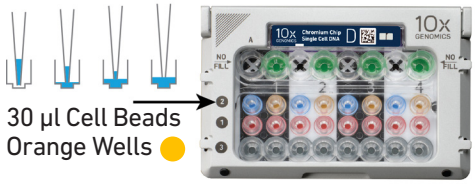
DO NOT add 50% glycerol solution to the top row, including the green Recovery Wells. DO NOT use any substitute for 50% glycerol solution.

c. Load Row Labeled 1
Dispense **35 µl** Reaction Mix into the bottom center of each well in **row labeled 1** without introducing bubbles.

Accurate input volume is critical for optimal performance.

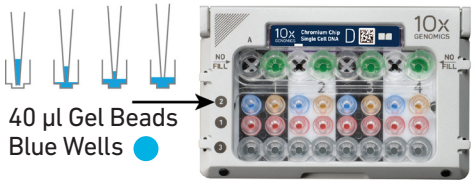


d. Load Cell Beads in Row Labeled 2 – Orange Wells
Pipette mix Cell Beads from step 2.3. Dispense **30 µl** Cell Beads into the orange wells in **row labeled 2** without introducing bubbles. DO NOT dispense into the blue wells.



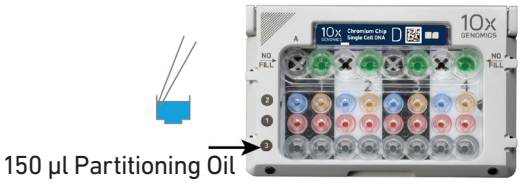
e. Prepare Gel Beads
Snap the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec.** Remove the Gel Bead strip and flick in a sharp, downward motion to ensure maximum recovery. Confirm there are no bubbles at the bottom of the tubes and liquid levels look even.

f. Load Gel Beads in Row Labeled 2 – Blue Wells
Puncture the foil seal of the Gel Bead tubes. Slowly aspirate **40 µl** Gel Beads. Dispense into the blue wells in **row labeled 2**. DO NOT dispense into the orange wells.

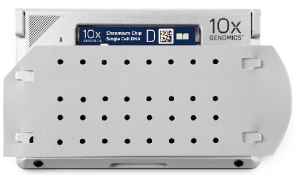


g. Load Row Labeled 3
Dispense **150 µl** Partitioning Oil into each well in **row labeled 3**.

Failure to add Partitioning Oil can damage the Chromium Controller.



h. Attach 10x Gasket
Align the notch with the top left-hand corner. Ensure the gasket holes are aligned with the wells. Avoid touching the smooth gasket surface. DO NOT press down on the gasket.



3.3 Run the Chromium Controller

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

b. Place the assembled chip with the gasket in the tray. Press the button to retract the tray.

c. Confirm the program on screen. Press the play button.

d. At completion of the run **(~4.5 min)**, the Controller will chime. **Immediately** proceed to the next step.

Usable Temperature Range
19-25°C (66-77°F)

3.4 Transfer GEMs

a. Place a PCR 8-tube strip on ice.

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

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

Expose Wells at 45 Degrees

d. Wait **30 sec**.

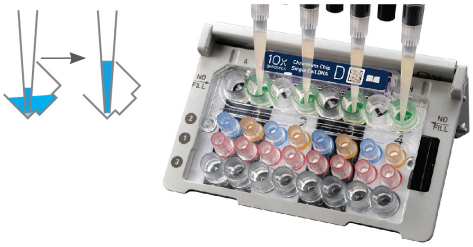
e. Check the volume in rows 1-3 and the recovery wells. Abnormal volumes indicate a clog.

Remove Partitioning Oil

f. Slowly remove **175 µl** Partitioning Oil from the lowest point of the green Recovery Wells. A variable small volume of GEMs may be aspirated.

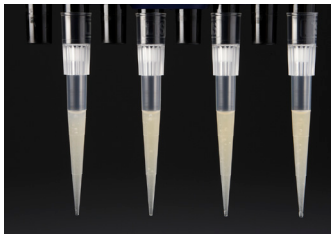
g. Slowly aspirate **125 µl** GEMs from the lowest points of the Recovery Wells without introducing bubbles.

Transfer GEMs



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

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



j. 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**.

3.5 GEM Isothermal 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
75°C	125 µl	~8 h 10 min
Step	**Temperature**	**Time**
1	30°C	03:00:00
2	16°C	05:00:00
3	65°C	00:10:00
4	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 4

Post GEM Incubation Cleanup & QC

4.1 Post GEM Incubation Cleanup – Dynabeads

4.2 Post GEM Incubation Cleanup – SPRIselect

4.3 Post GEM QC

4.0 Post GEM Incubation Cleanup & QC

GET STARTED!					
Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature		**Additive A**	220074	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
		Dynabeads MyOne SILANE	2000048	Vortex thoroughly to resuspend beads immediately before use.	4°C
		Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
		Agilent Bioanalyzer High Sensitivity Kit	-	Manufacturer's recommendations.	-
Thaw at 65°C		**Buffer Sample Clean Up 1**	220020	Thaw for 10 min at 65°C at max speed on a thermomixer. Verify there are no visible crystals. Cool to room temperature.	–20°C
Obtain		**Recovery Agent**	220016	-	Ambient
		Qiagen Buffer EB	-	Manufacturer's recommendations.	-
		Bio-Rad 10% Tween 20	-	Manufacturer's recommendations.	-
		10x Magnetic Separator A	2000067	-	Ambient
		10x Magnetic Separator	230003	-	Ambient
		Prepare 80% Ethanol Prepare 10 ml for 8 reactions	-	Prepare fresh.	-

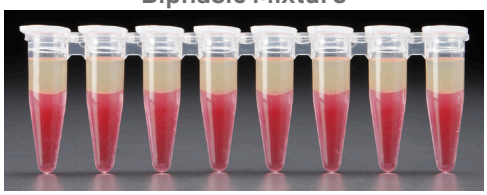
4.1 Post GEM Incubation Cleanup – Dynabeads

a. Add **125 µl** Recovery Agent to each sample at **room temperature**.

b. Mix by gently inverting 25x.

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

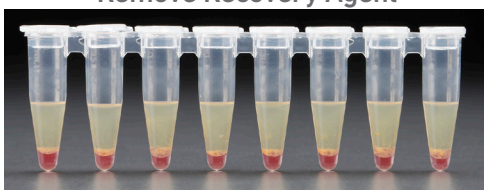
Biphasic Mixture



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

d. Slowly remove **135 µl** Recovery Agent/ Partitioning Oil (pink) from the bottom of the tube. A small volume of pink solution may remain in the tube. DO NOT aspirate any aqueous sample.

Remove Recovery Agent



If aqueous solution is aspirated, return the solution to the tube strip, reduce removal volume by **5 µl**, and reattempt removal.

e. Prepare Dynabeads Cleanup Mix. Vortex and add **162 µl** to each sample.

Add Dynabeads Cleanup Mix

Dynabeads Cleanup Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Buffer Sample Clean Up 1	220020	140.0	616.0	1232.0
Dynabeads MyOne SILANE Vortex thoroughly before adding to the mix.	2000048	16.0	70.4	140.8
Additive A	220074	6.0	26.4	52.8
Total	-	**162.0**	**712.8**	**1425.6**

f. Pipette mix 5x. DO NOT cap the tubes.

g. Incubate **10 min** at **room temperature**. Pipette mix again at **~5 min** after start of incubation to resuspend settled beads.

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

Elution Solution I *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Buffer EB	-	94.0	413.6	827.2
10% Tween 20	-	1.0	4.4	8.8
Additive A	220074	5.0	22.0	44.0
Total	-	**100.0**	**440.0**	**880.0**

i. At the end of **10 min** incubation, place on magnet **A** until the solution clears.

j. Remove the supernatant.

k. Add **250 µl** freshly prepared 80% ethanol to the pellet while on magnet **A**. Wait **30 sec**.

l. Remove the ethanol.

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

n. Remove the ethanol.

o. Centrifuge briefly. Place on 10x Magnetic Separator at **Low** position.

TIPS

Use the two-position 10x Magnetic Separator, which is different from the 10x Magnetic Separator A.

p. Remove remaining ethanol.

q. Remove from the magnet. **Immediately** add **50.5 µl** Elution Solution I to avoid clumping.

r. Pipette mix (pipette set to 40 µl) without introducing bubbles.

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

t. Centrifuge briefly. Place on the magnet•**Low** until the solution clears.

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

4.2 Post GEM Incubation Cleanup – SPRIselect

This step includes **two consecutive SPRIselect Cleanups**.

a. Prepare Elution Solution II. Vortex and centrifuge briefly.

Elution Solution II *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Buffer EB	-	100	440	880
Additive A	220074	3	13.2	26.4
Total	-	**103**	**453.2**	**906.4**

b. Vortex the SPRIselect reagent until fully resuspended. Add **50 µl** SPRIselect reagent to each sample. Pipette mix thoroughly.

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

d. Centrifuge briefly. Place on the magnet•**High** until the solution clears.

e. Remove the supernatant.

f. Add **125 µl** 80% ethanol to the pellet. Wait **30 sec**.

g. Remove the ethanol.

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

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

j. Remove any remaining ethanol.

k. Remove the tube strip from the magnet. **Immediately** add **51.5 µl** Elution Solution II to avoid clumping.

l. Pipette mix (pipette set to 40 µl) without introducing bubbles.

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

n. Centrifuge briefly. Place on the magnet•**Low** until the solution clears.

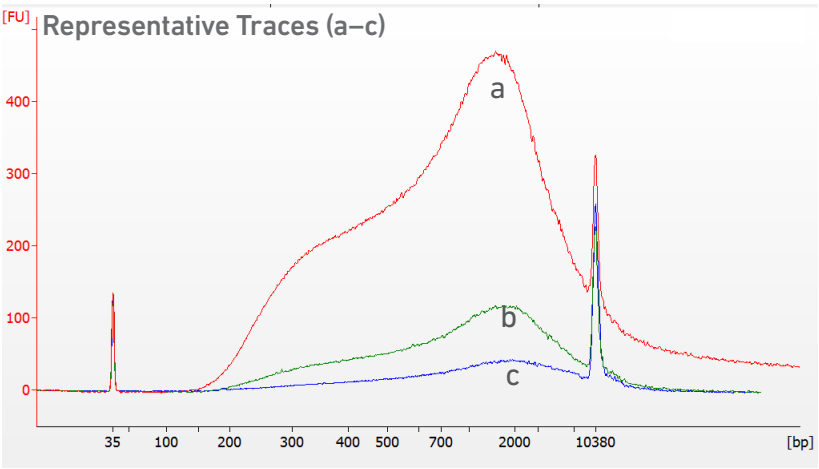
o. Transfer **51 µl** sample to a new tube strip.

p. Repeat steps b – o for a total of 2 SPRIselect Cleanups.

q. Store at **4°C** for up to **72 h** or at **–20°C** for up to **2 weeks,** or proceed to the next step.

4.3 Post GEM QC

a. Run **1 µl** sample on the Agilent Bioanalyzer High Sensitivity DNA chip. Representative traces (a-c) from three samples.



Step 5

Library Construction

5.0 Library Construction

GET STARTED!				
Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	**End Repair and A-tailing Buffer**	220120	Equilibrate to room temperature 30 min before using.	–20°C
	Forward PCR Primer	220124	-	–20°C
	Adaptor Mix	220026	-	–20°C
	Ligation Buffer	220109	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
	Chromium i7 Sample Index Plate	220103	-	–20°C
	Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
	Agilent DNA 1000 kit If used for QC	-	Manufacturer's recommendations.	-
Place on Ice	**End Repair and A-tailing Enzyme**	220121	-	–20°C
	DNA Ligase	220110		–20°C
	Amplification Master Mix	220125		–20°C
	KAPA Library Quantification Kit for Illumina Platforms	-	Manufacturer's recommendations.	-
Obtain	**Qiagen Buffer EB**	-	-	Ambient
	10x Magnetic Separator	230003	See Tips & Best Practices.	Ambient
	Prepare 80% Ethanol Prepare 10 ml for 8 reactions	-	Prepare fresh.	Ambient

5.1 End Repair & A-tailing

a. Prepare End Repair and A-tailing Mix. Pipette mix thoroughly and centrifuge briefly.

End Repair and A-tailing Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Nuclease-free Water	-	2.5	11.0	22.0
End Repair and A-tailing Buffer	220120	7.5	33.0	66.0
End Repair and A-tailing Enzyme	220121	15.0	66.0	132.0
Total	-	**25**	**110**	**220**

b. Add **25 µl** End Repair and A-tailing Mix to **50 µl** sample. Pipette mix thoroughly and centrifuge briefly.

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

Lid Temperature	Reaction Volume	Run Time
85°C	75 µl	~1 h
Step	**Temperature**	**Time**
End Repair	20°C	00:30:00
A-tailing	65°C	00:30:00
Hold	4°C	Hold

5.2 Adaptor Ligation

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

Adaptor Ligation Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Ligation Buffer	220109	22.0	96.8	193.6
DNA Ligase	220110	11.0	48.4	96.8
Adaptor Mix	220026	2.5	11.0	22.0
Total	-	**35.5**	**156.2**	**312.4**

b. Add **35.5 µl** Adaptor Ligation Mix to **75 µl** sample. Pipette mix thoroughly (pipette set to 90 µl) and centrifuge briefly.

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

Lid Temperature	Reaction Volume	Run Time
30°C	110 µl	15 min
Step	**Temperature**	**Time**
1	20°C	00:15:00
2	4°C	Hold

5.3 Post Ligation Cleanup – SPRIselect

a. Vortex to resuspend SPRIselect reagent. Add **88 µl** SPRIselect reagent to each sample. Pipette mix thoroughly.

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

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

d. Remove the supernatant.

e. Add **250 µl** 80% ethanol. 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.

j. Remove from the magnet. **Immediately** add **50.5 µl** Buffer EB. Pipette mix thoroughly.

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

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

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

n. Vortex to resuspend SPRIselect reagent. Add **50 µl** SPRIselect reagent to each sample. Pipette mix.

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

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

q. Remove the supernatant.

r. Add **125 µl** 80% ethanol to the pellet. Wait **30 sec**.

s. Remove the ethanol.

t. **Repeat** steps r and s for a total of 2 washes.

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

v. Remove remaining ethanol. **Immediately** add **40.5 µl** Buffer EB. Pipette mix thoroughly.

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

x. Centrifuge briefly. Place on the magnet•**Low** until solution clears.

y. Transfer **40 µl** sample to a new tube strip. **Immediately** proceed to the next step.

5.4 Sample Index PCR

Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run.

a. Prepare Sample Index PCR Mix.

Sample Index PCR Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Amplification Master Mix	220125	50	220	440
Forward PCR Primer	220124	5	22	44
Total	-	**55**	**242**	**484**

b. Add **55 µl** Sample Index PCR Mix to **40 µl** sample. Pipette mix and centrifuge briefly.

c. Add **5 µl** of an individual Chromium i7 Sample Index to each well. Record assignment. Pipette mix and 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**
1	98°C	00:00:45
2	98°C	00:00:20
3	54°C	00:00:30
4	72°C	00:00:30
5	Go to step 2, 11-13X (Total 12 or 14 cycles) 12 cycles when targeted cell number is >1000 14 cycles when target cell number is ≤1000	
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.

5.5 Post Sample Index Double Sided Size Selection – SPRIselect

a. Vortex to resuspend SPRIselect reagent. Add **50 µl** SPRIselect reagent to each sample. Pipette mix.

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

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

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

DO NOT discard supernatant.

e. Vortex to resuspend SPRIselect reagent. Add **40 µl** SPRIselect reagent to each sample. Pipette mix.

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

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

h. Remove the supernatant.

i. 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.

n. Remove from the magnet. **Immediately** add **35.5 µl** Buffer EB. Pipette mix.

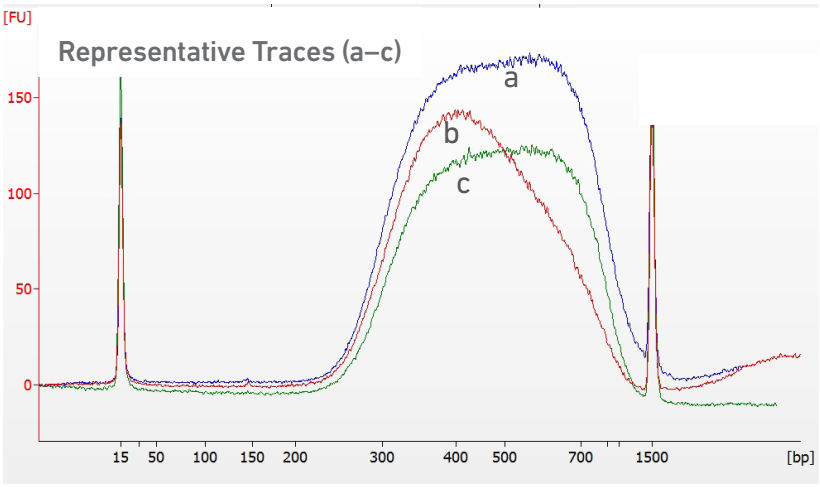
o. Incubate **2 min** at **room temperature**.

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

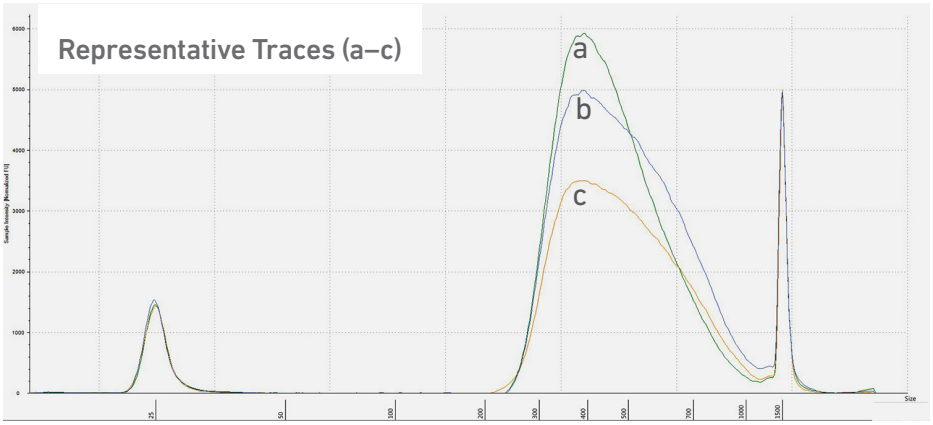
q. Transfer **35 µl** sample to a new tube strip.

5.6 Post Library Construction QC

a. EITHER Run **1 µl** sample on the Agilent Bioanalyzer DNA 1000 chip to determine fragment size. Representative traces (a-c) from three samples.



b. OR Run **1 µl** sample on the Agilent TapeStation D1000 ScreenTape to determine fragment size. Representative traces (a-c) from three samples.



5.7 Post Library Construction Quantification

a. Thaw KAPA Library Quantification Kit for Illumina Platforms.

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

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

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

d. Dispense **16 µl** Quantification Master Mix for sample dilutions and DNA Standards into a tube strip.

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

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

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

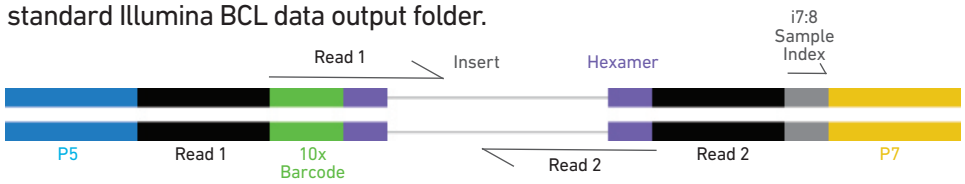
g. Follow the manufacturer's recommendations for qPCR-based quantification. For library quantification for sequencer clustering, determine the concentration using a fixed insert size of 550 bp.

Sequencing

6

Sequencing Libraries

Chromium Single Cell DNA libraries comprise standard Illumina paired-end constructs which begin with P5 and end with P7. Sequencing these libraries produces a standard Illumina BCL data output folder.



The BCL data for Single Cell DNA libraries includes:

- Paired-end Read 1 containing the 16 bp 10x Barcode and gDNA in the insert
- Read 2 containing gDNA in the insert
- 8 bp sample index in the i7 read

Cell Ranger DNA performs demultiplexing and leverages the 10x Barcodes to group read pairs and associate them to individual cells. These reads are mapped to their reference standards with their relative occurrence in each genomic region yielding insights into copy number variation (CNV).

Illumina Sequencer Compatibility

The compatibility of the listed sequencers has been verified by10x Genomics. Some variation in assay performance is expected based on sequencer choice. For more information about performance variation, visit the 10x Genomics Support website.

- NextSeq 500/550 (High Output)
- HiSeq 2500 (Rapid Run)
- HiSeq 3000/4000
- HiSeq X
- NovaSeq

Sample Indices

Each sample index in the Chromium i7 Sample Index Kit (PN-120262) is a mix of 4 different sequences to balance across all 4 nucleotides. If multiple samples are pooled in a sequence lane, the sample index name (i.e. the Chromium i7 Sample Index plate well ID) is needed in the sample sheet used for generating FASTQs with "cellranger-dna mkfastq".

Sequencing Depth & Run Parameters

Sequencing Depth	750,000 read pairs per cell[1]
Sequencing Type	Paired-end, single indexing[2]
Sequencing Read	Recommended Number of Cycles[3]
Read 1	100 or 150 cycles
i7 Index	8 cycles
i5 Index	0 cycles
Read 2	100 or 150 cycles

1. The recommended sequencing depth enables 2 Mb CNV event detection per single cell. Lower sequencing depth will reduce the resolution of CNV calling.
2. Although single indexing is recommended, if a dual-index configuration is used, use bcl2fastq's --use-bases-mask or mkfastq's --ignore-dual-index option to ignore the I2 read.
3. Sample index reads must not be shorter than indicated. Any read can be longer than recommended. Additional bases in sample index reads must be trimmed using "cellranger-dna mkfastq" or Illumina's "bcl2fastq" prior to further analysis.

Library Loading

Once quantified and normalized, Single Cell DNA libraries should be denatured and diluted according to the table below. Consult the Sequencing Metrics and Base Composition of Sequencing Reads of Chromium Single Cell DNA Libraries (Document CG000163), available at the 10x Genomics Support website, for more information.

Instrument	Loading Concentration (pM)	PhiX (%)
NextSeq 500	1.7	1
HiSeq 2500 (RR)	10	1
HiSeq 4000	240	1
HiSeq X	240	1
NovaSeq	300	1

Library Pooling

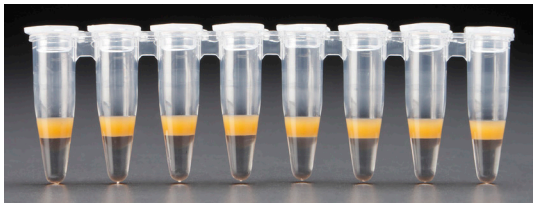
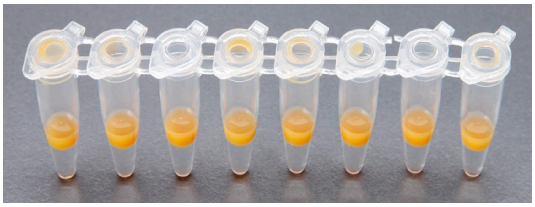
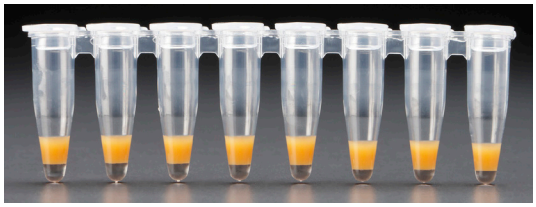
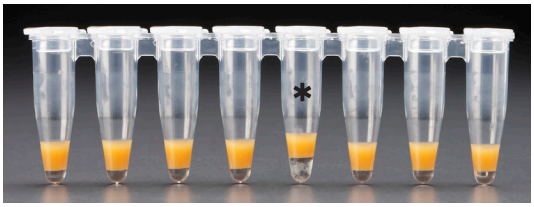
Pooling dissimilar libraries may compromise the ability to pool effectively due to differences in insert sizes.

Troubleshooting

7

7.0 Troubleshooting

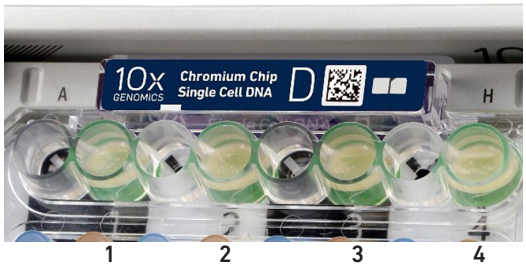
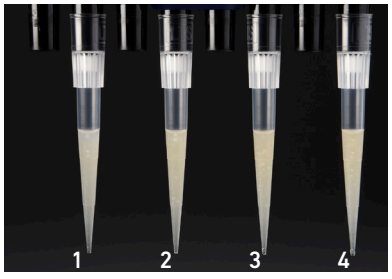
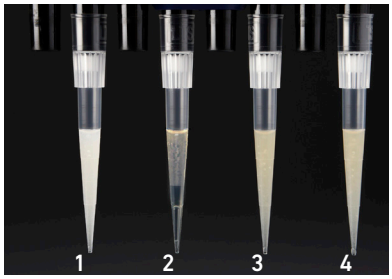
7.1 Cell Beads

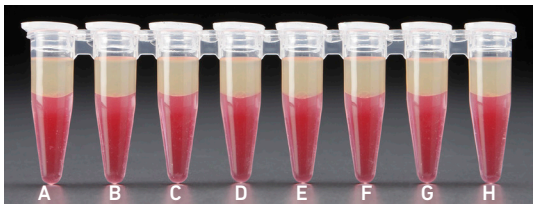
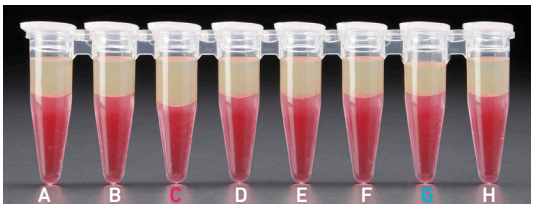
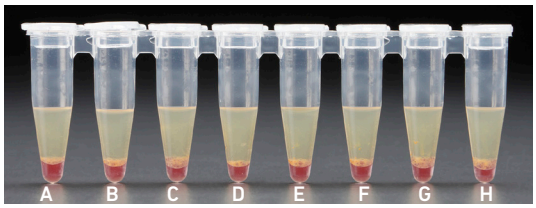
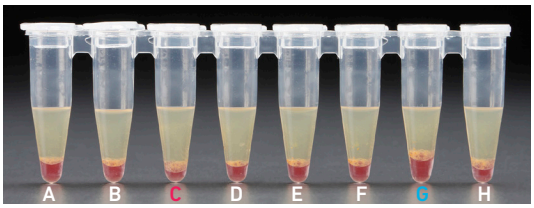
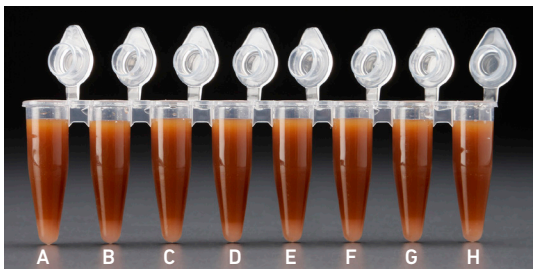
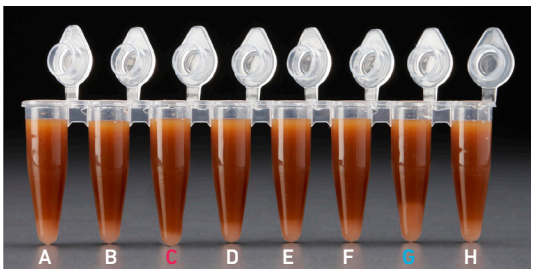
STEP	NORMAL	PROBLEM & SOLUTION
1.1 d Mixing the Cell Bead Mix	Cell Bead Mix is uniform with no visible magnetic bead clumps.	Cell Bead Mix has magnetic bead clumps. **Solution:** Using a pipette tip, aspirate from the bottom of the tube and dispense at the liquid surface multiple times to break the magnetic bead clumps.
1.1 d Loading Cell Bead Mix into Chip C	Dispense **23 µl** Cell Bead Mix into the bottom center of each well in **row labeled 1** without introducing bubbles.	**<23 µl** Cell Bead Mix aspirated in the pipette tip for dispensing. **Solution:** Using a pipette tip, push down Cell Bead Mix stuck to the sidewalls of the tube. Slowly aspirate from the bottom of the tube holding the pipette tips in the solution for **~2 sec** after the plunger is fully released.
1.2 i Transferring tubes to the thermomixer	 Cell Beads are uniform and not stuck on the sidewalls or caps of the tubes.	 Cell Beads are stuck on the sidewalls and caps of the tubes. DO NOT flick, shake or vortex the tubes when transferring to the thermomixer. **Solution:** Centrifuge briefly to recover beads.
2.1 b After removal of Partitioning Oil	 **5-10 µl** Partitioning Oil remaining in the tubes.	 **>10 µl** Partitioning Oil remaining in tube*. **Solution:** Remove oil from the tube* until remaining oil volume is **5-10 µl**.

STEP	NORMAL	PROBLEM & SOLUTION
2.2 n Removal of PBS from the bottom of the tubes, retaining the Cell Beads	 PBS removed uniformly, retaining Cell Beads.	 Variable removal of PBS. **Solution:** Add or remove PBS until uniform.
2.2 s Cell Beads migrating to the magnets	 Cell Beads migrate to the magnets uniformly.	 Cell Beads migrate to the magnets variably. **Solution:** Use pipette tip to gently mix Cell Beads to enable uniform migration to the magnets. 
2.3 d Pass Cell Bead suspension through Flowmi filter	Cell Bead suspension passes through the Flowmi filter into a new 1.5-ml microcentrifuge tube.	Flowmi filter clogs while passing the Cell Bead suspension. **Solution:** Discard clogged Flowmi filter and proceed with a new Flowmi filter. Some loss in Cell Bead suspension volume is expected.

7.2 GEMs

STEP	NORMAL	PROBLEM & SOLUTION
3.2 d Loading Cell Beads in Chromium Chip D	**30 µl** Cell Beads loaded in Chip D.	Less than **30 µl** Cell Beads available for loading. **Solution:** Return Cell Beads to the tube, centrifuge briefly. If volume is still **<30 µl**, load available volume (not **<25 µl**). If volume **<25 µl**, add CB Buffer to bring volume to **25 µl**, pipette mix, and load the entire volume in Chip D. Loading less than **<30 µl** Cell Beads may reduce cell recovery efficiency. **Additional Tips:** To achieve optimal Cell Bead volume for loading in Chip D, accurate pipetting is critical in steps 2.3 g-j. • Pipette slowly and accurately using calibrated pipettes with compatible pipette tips. • After aspirating liquid, check pipette tips for air pockets/bubbles. • In step 2.3.i, a variable small volume of Cell Beads may be aspirated. All samples remaining in the tube strip should have similar meniscus levels. Briefly centrifuge tube strip containing Cell Beads before loading in Chip D. Gently remove tube strip form centrifuge to avoid splashing the Cell Beads.

STEP	NORMAL	REAGENT CLOGS & WETTING FAILURES
3.4 e After Chip D is removed from the Controller and the wells are exposed	 All 4 green Recovery Wells are similar in volume and opacity.	 Recovery Well 1 indicates a reagent clog. Recovery Well 2 indicates a wetting failure. Recovery Wells 3 and 4 are normal. The opacity of the Recovery Wells 1 and 2 is different from the wells 3 and 4.
3.4 g Transfer GEMs from Chip D Recovery Wells	  All liquid levels are similar in volume and opacity without air trapped in the pipette tips.	  Pipette tip 1 (lighter color) indicates a reagent clog. Pipette tip 2 indicates a wetting failure.

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

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.

7.3 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 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 when the 10x Gasket installation was forgotten, install and try again. In the case when 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, the Chromium Chip must be discarded. **Do not try running this Chromium Chip again as this may 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 when 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.

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