Chromium™

Genome Reagent Kits v2 User Guide

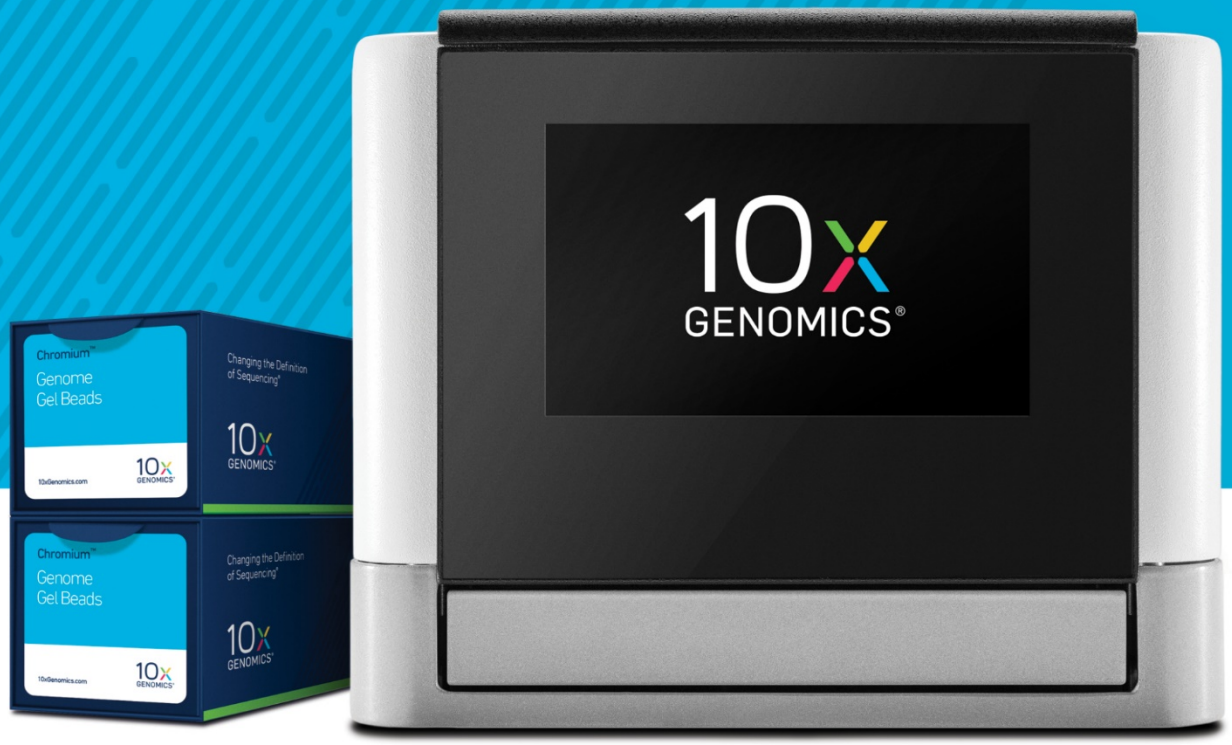
FOR USE WITH

Chromium™ Genome Library Kit & Gel Bead Kit v2, 16 rxns PN-120258

Chromium™ Genome HT Library Kit & Gel Bead Kit v2, 96 rxns PN-120261

Chromium™ Genome Chip Kit v2, 48 rxns PN-120257

Chromium™ i7 Multiplex Kit PN-120262



10x GENOMICS®

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Notices

Manual Part Number

CG00043 Rev B

Legal Notices

Customer Information and Feedback

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Chromium™ Genome Reagent Kits v2 – Components

CRITICAL!

*Parts from Chromium™ Genome Reagents Kits v2 are **NOT** interchangable with parts from earlier Chromium Genome Reagent Kits, despite the same or similar names.*

Chromium™ Genome Library & Gel Bead Kit v2, 16 rxns PN-1000017			
Product	Description	#	Part Number
Chromium™ Genome Library Kit v2 (store at –20°C)			120255
Reagents Module 1	Denaturing Agent	1	220072
	Genome Enzyme Mix	1	220122
	Genome Reagent Mix	1	220123
	Additive A	2	220074
	Control DNA	1	220045
	Buffer Sample Clean Up 1	2	220020
Reagents Module 2	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
Chromium™ Genome Gel Bead Kit (store at –80°C)			120214
	Chromium™ Genome Gel Beads	2	220058

Chromium™ Genome HT Reagent Kits v2 – Components

CRITICAL! *Parts from Chromium™ Genome Reagents Kits v2 are **NOT** interchangable with parts from earlier Chromium Genome Reagent Kits, despite the same or similar names.*

Chromium™ Genome HT Library & Gel Bead Kit, 96 rxns PN-1000018			
Product	Description	#	Part Number
Chromium™ Genome HT Library Kit v2 (store at –20°C)			120259
Reagents Module 1	Denaturing Agent	2	220072
	Genome Enzyme Mix	2	220113
	Genome Reagent Mix	2	220114
	Additive A	2	220093
	Buffer Sample Clean Up 1	2	220094
Reagents Module 2	End Repair and A-tailing Buffer	1	220115
	End Repair and A-tailing Enzyme	1	220116
	Ligation Buffer	1	220117
	DNA Ligase	1	220118
	Adaptor Mix	1	220099
	Amplification Master Mix	1	220119
	Forward PCR Primer	1	220101
Chromium™ Genome HT Gel Bead Kit (store at –80°C)			120247
	Chromium™ Genome Gel Bead Plate (96 rxn)	1	220102

Chromium™ Genome Reagent Kits

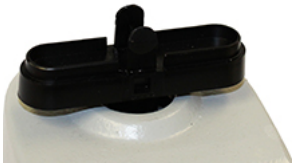
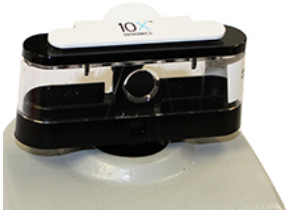
CRITICAL!

*Parts from Chromium™ Genome Reagents Kits v2 are **NOT** interchangable with parts from earlier Chromium™ Genome Reagent Kits, despite the same or similar names.*

Chromium™ Genome Chip Kit, 48 rxns PN-120257			
Product	Description	#	Part Number
Chromium™ Genome Chip Kit v2, 48 rxns (store at ambient temperature)			120257
	Genome Chips	1	230028
	Gaskets	1	370017
	Partitioning Oil	4	220088
	Recovery Agent	6	220016

Chromium™ Multiplex Kit, 96 rxns PN-120262			
Product	Description	#	Part Number
Chromium™ i7 Multiplex Kit, 96 rxns (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 most standard laboratory vortexers and enables the use of the 10x Vortex Clip and to vortex Gel Bead Strips. 	330002
10x™ Vortex Clip	The 10x Vortex Clip coupled with the 10x Vortex Adapter enables users to vortex 8-tube strips with ease. 	230002
10x™ Chip Holder	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. 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. 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.  	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

The items in the table below have been validated by 10x Genomics® and are highly recommended for Genome workflow, training, and system operations. USA Scientific, Eppendorf, and Thermo Fisher PCR 8-tube strips have also been validated. Substituting materials may adversely affect system performance. Either Bioanalyzer, TapeStation, or Fragment Analyzer™ are needed for DNA quality control. Wide-bore tips are required for HMW gDNA handling.

Supplier	Description	Part Number (US)
Plastics		
Eppendorf	PCR Tubes 0.2 ml 8-tube strips	0030124286
	DNA LoBind Tubes, 1.5 ml	022431021
	DNA LoBind Tubes, 2.0 ml	022431048
	DNA LoBind Tube, 5.0 ml	0030108310
USA Scientific	TempAssure PCR 8-tube strip *(alternate to Eppendorf OR Thermo Fisher Scientific product)*	1402-4700
Thermo Fisher Scientific	MicroAmp® 8-Tube Strip, 0.2 ml *(alternate to Eppendorf or USA Scientific product)*	N8010580
	MicroAmp® 8 -Cap Strip, clear	N8010535
Kits & Reagents		
Qiagen	Buffer EB	19086
Thermo Fisher Scientific	DynaBeads® MyOne™ Silane Beads	37002D
	Nuclease-Free Water	AM9937
Sigma	Ethanol, Pure (200 Proof, anhydrous)	459836-500ML
Beckman Coulter	SPRIselect Reagent Kit	B23318
Bio-Rad	10% Tween 20	1610781
Ricca Chemical Company	Glycerin (glycerol), 50% (v/v) Aqueous Solution	3290-32
Equipment		
Rainin	Tips LTS 200UL Filter RT-L200FLR	17007961
	Pipet-Lite Multi Pipette L8-50XLS+	17013804
	Pipet-Lite Multi Pipette L8-200XLS+	17013805
VWR	Vortex Mixer	10153-838
	Divided Polystyrene Reservoirs	41428-958

Table continued in next page

Additional Kits, Reagents & Equipment

Table continued from previous page

Quantification & Quality Control		
Agilent	2100 Bioanalyzer Laptop Bundle	G2943CA
	High Sensitivity DNA Kit	5067-4626
	DNA 1000 Kit	5067-1504
	4200 TapeStation	G2291aa
	D1000 ScreenTape	5067-5582
	D1000 Reagents	5067-5583
	High Sensitivity D1000 ScreenTape	5067-5584
	High Sensitivity D1000 Reagents	5067-5585
Thermo Fisher Scientific	Qubit® 3.0 Fluorometer	Q33216
	Qubit® dsDNA HS Assay Kit	Q32854
	Qubit® Assay Tubes	Q32856
Advanced Analytical®	Fragment Analyzer™ Automated CE System – 12 cap	FSv2-CE2F
	Fragment Analyzer™ Automated CE System – 48/96 cap	FSv2-CE10F
	High Sensitivity NGS Fragment Analysis Kit	DNF-474
KAPA Biosystems	Illumina® Library Quantification Kit	KK4824

Not all of these instruments and reagents are required.

Choose among Bioanalyzer, TapeStation, and Fragment Analyzer based on availability and preferences.

Additional Kits, Reagents & Equipment

The following are additional and alternative kits, reagents, and equipment recommended by 10x Genomics® and contribute to optimal system performance. If using plates, Eppendorf twin.tec® PCR plates are recommended to ensure stability of GEM emulsions, and the specific model should be selected based on compatibility with thermal cycler in use.

Supplier	Description	Part Number (US)
Plastics		
Eppendorf	twin.tec® 96-Well PCR Plate Semi-skirted twin.tec® 96-Well PCR Plate Divisible, Unskirted twin.tec® 96-Well PCR Plate Unskirted Heat Sealing Foil, PCR clean *(alternate to Bio-Rad product)*	0030129326 2231000209 0030133390 0030127854
Bio-Rad	Optical Flat 8-Cap Strips Microseal 'B' Adhesive Seals Hard-Shell Low-Profile Thin-Wall 96-Well Skirted PCR Plates Pierceable Foil Heat Seal *(if PCR plates used)*	TCS0803 MSB1001 HSP9665 1814040
Equipment		
Eppendorf	ThermoMixer C® SmartBlock 1.5 ml, Thermoblock for 24 Reaction Vessels *(alternatively, use a temperature-controlled Heat Block)*	5382000015 5360000038
Bio-Rad	PX1™ PCR Plate Sealer *(if PCR plates used)*	1814000
Rainin	Tips LTS 20UL Filter RT-L10FLR Tips LTS 200UL Filter RT-L200FL Tips LTS 1ML Filter RT-L1000FLR 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+	17007957 17007961 17007954 17014393 17014388 17014392 17014384 17014391 17014382 17013802 17013803
-	qPCR instrument and compatible consumables	

Choose specific plate based on thermal cycler compatibility

Recommended Thermal Cyclers

Thermal cyclers used with the Genome Protocol must support uniform heating of 125 µl emulsion volumes. Thermal cyclers recommended for use with the Genome 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 Genome Reagent Kit Protocol

Stepwise Objectives

Steps & Timing

The Genome Reagent Kit Protocol – Stepwise Objectives

Step 1 – HMW gDNA Extraction

The Chromium™ Genome Protocol generates long-range information across the length of individual DNA molecules. For this solution, starting the process with high molecular weight (HMW) genomic DNA (gDNA) results in better performance, such as increased haplotype phase block length and ability to call structural variants. Optimal performance has been characterized on input gDNA with a mean length greater than 65 kb, and this protocol outlines the extraction of HMW gDNA with optimal size from live cells.

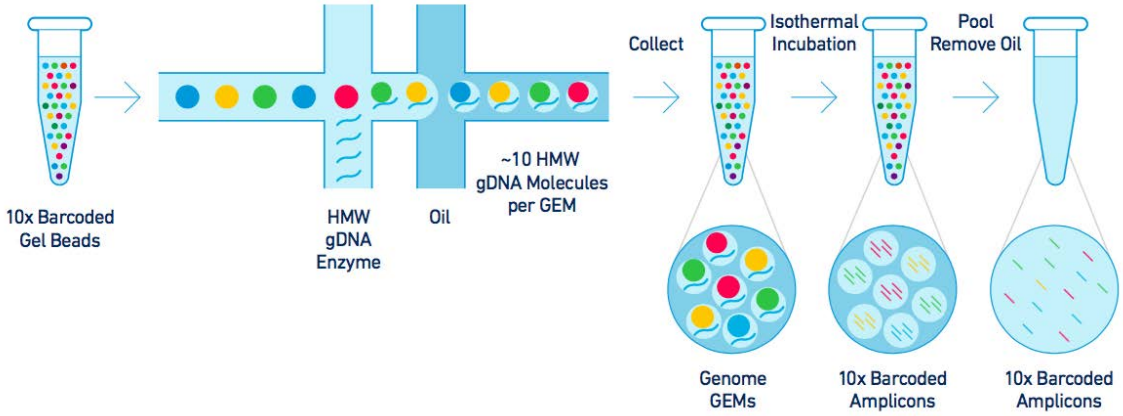
Step 2 – GEM Generation & Barcoding

In the microfluidic Genome Chip, a library of Genome Gel Beads is combined with an optimal amount of HMW template gDNA in Master Mix and partitioning oil to create Gel Bead-In-EMulsions (GEMs). Each Gel Bead is functionalized with millions of copies of a 10x™ Barcoded primer and the barcoding reaction takes place in volumes on the order of 100 picoliters.

Approximately 1.25-1.5 ng (~375 – 450 haploid genome equivalents) of template gDNA is partitioned across over 1 million GEMs and consequently only a small number of genomic equivalents are loaded in each GEM (~500 kb after accounting for system losses, or 0.02% of the haploid human genome). With such dispersion of template, it is highly unlikely that two distinct gDNA molecules that cover the same locus with opposing haplotypes will have the same 10x Barcode. Loading the correct amount of gDNA into the system during GEM generation is critical to optimal performance. It is also important to note only approximately 50% of loaded gDNA is incorporated into the final sequencing library – the remainder is left behind in the microfluidic Genome Chip.

Upon dissolution of the Genome Gel Bead in the GEM, primers containing (i) an Illumina® R1 sequence (Read 1 sequencing primer), (ii) a 16 nt 10x Barcode, and (iii) a 6 nt random primer sequence are released.

Isothermal incubation of the GEMs produces barcoded fragments ranging from a few to several hundred base pairs. After incubation, the GEMs are broken and the pooled fractions are recovered.



Step 3 – Post GEM 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 appropriate DNA size range for library preparation.

Step 4 – Library Construction

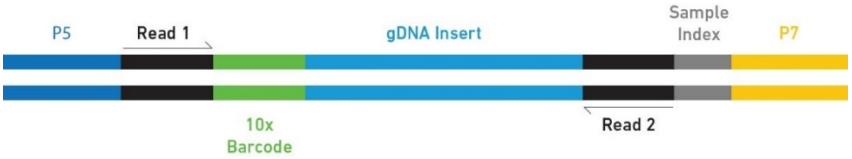
Read 1 sequence and the 10x™ Barcode are added to the molecules during the GEM incubation. P5 and P7 primers, Read 2, and Sample Index are added during library construction via end repair, A-tailing, adaptor ligation, and amplification. The final libraries contain the P5 and P7 primers used in Illumina® bridge amplification.

Step 5 – Sequencing Libraries

The Chromium™ Genome Protocol produces Illumina sequencing-ready libraries. This section describes the steps required to ensure the successful sequencing of Genome libraries to deliver the full value of the Chromium Genome Solution.

Genome libraries comprise standard Illumina paired-end constructs which begin and end with P5 and P7. The 16 bp 10x Barcode is 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.

Final Library Construction:



Library Analysis – Chromium™ Software

Built upon widely accepted aligners and variant callers, the Long Ranger™ analysis pipelines leverage the Linked-Reads created by 10x Barcodes to yield insight into megabase-scale haplotype phasing, structural variant detection and phasing, and greater accuracy in SNP and indel calling. The Supernova™ Assembler leverages the unique properties of Linked-Read data to reconstruct the genome, without the need for a reference. The Loupe™ family of visualization applications brings clarity to the novel biological insights made possible by the Chromium System.

The Genome Reagent Kit Protocol – Steps & Timing

Steps and timing are applicable to both the Genome v2 (16 rxns) Kit and the Genome HT (96 rxns) Kit.

		Bench Time	Instrumentation Time	Stop & Store Options
Day 1		gDNA Extraction 1 - 1.5 h		STOP 4°C ≤2 weeks or –20°C ≤6 months
	1 h	Input gDNA Quantification & Dilution ~ 1.5 h 1 - 2 h (8 samples)		
	2 h	Reagent Prep - 20 min Loading Genome Chip - 10 min		
	3 h	Transferring GEMs - 3 min	GEM Generation - 20 min	
	4 h		GEM Isothermal Incubation - 3 h	
	5 h			
	6 h			STOP 4°C ≤72 h or –20°C ≤2 weeks
	7 h	Post GEM Recovery - 10 min Cleanup - Silane Beads - 35 min Cleanup - SPRIselect - 20 min		STOP 4°C ≤72 h or –20°C ≤2 weeks
Day 2	1 h	End Repair & A-tailing Prep - 10 min	QC - 50 min	
	2 h		End Repair & A-tailing Incubation - 1 h	
	3 h	Adaptor Ligation Prep - 10 min Cleanup - SPRIselect - 20 min Sample Index PCR Prep - 10 min	Adaptor Ligation Incubation - 15 min	
	4 h	Double Sided SPRIselect Sizing - 25 min	Sample Index PCR - 30 min	STOP 4°C ≤72 h STOP 4°C ≤72 h or –20°C long-term
	5 h		QC - 50 min qPCR Quantification*	

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

Protocol Step 1

HMW gDNA Extraction

Extracting high molecular weight genomic DNA to maximize application performance using Linked-Reads

1

1. HMW gDNA Extraction

Tips

High Input DNA Length Results in Optimal Performance

The Chromium™ Genome Protocol generates long-range information across the length of individual DNA molecules. Starting the process with HMW gDNA will typically result in better application performance, such as increased haplotype phase block length and ability to call structural variants. Optimal performance has been characterized on input gDNA with a mean length greater than 50 kb.

Best Practices for Handling HMW gDNA

The following tips are designed to maintain the integrity of HMW gDNA through extraction:

- Never vortex tubes containing HMW gDNA
- Avoid repeated freeze/thaw cycles of HMW gDNA
- Extracted HMW gDNA samples (>10 ng/µl) can be stored at 4°C for up to 2 weeks, or at –20°C for up to 6 months
- Prepare multiple aliquots at –20°C if a stored HMW gDNA solution will be analyzed multiple times
- Use nuclease-free reagents and consumables

Getting Started! – Both Genome v2 (16 rxns) & HT v2 (96 rxns) Kits

The Chromium™ Genome Protocol supports the extraction of DNA from cultured cells using the Qiagen MagAttract HMW Kit (PN-67653), with minor modifications. Please refer to the manufacturer's brochure (Qiagen MagAttract HMW DNA Kit Handbook) for reagent preparation, storage, and troubleshooting.

HMW gDNA Extraction

a) Dispense 1 x 10^6 live cells per extraction into a 2 ml microcentrifuge tube. Centrifuge for **5 sec** at **15,000 x g**.

b) Carefully aspirate and discard media, leaving only the cell pellet behind.

c) Add **200 µl** room temperature (15–25°C) PBS buffer. Resuspend cell pellet by inverting 20 times.

d) Add **20 µl** Proteinase K. Mix by inverting the tube 5 times.

NOTE

Add and mix Proteinase K before proceeding. Do not combine steps d and e.

e) Add **4 µl** RNAse A and **150 µl** Buffer AL to the sample. Mix by pulse-vortexing 3 times at the highest speed setting. Incubate the sample for **30 min** at **25°C** and then centrifuge briefly.

f) Vortex the MagAttract® Suspension G for **1 min** and add **15 µl** to the sample.

NOTE

If this is the first time using MagAttract Suspension G, increase the vortexing time to 3 min.

g) Add **280 µl** Buffer MB. Incubate at **25°C** and **1400 rpm** for **3 min**.

h) Centrifuge the tube briefly and place on a DynaMag™-2 Magnetic Rack for **1 min** to allow bead capture. Remove and discard the supernatant. Take care not to disturb the bead pellet.

i) Remove the sample from the magnetic rack. Add **700 µl** Buffer MW1 directly to the bead pellet. Incubate at **25°C** and **1400 rpm** for **1 min**.

Repeat

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

k) Centrifuge the tube briefly and place on the magnetic rack for **1 min** to allow bead capture. Remove and discard the supernatant. Take care not to disturb the bead pellet.

l) Remove the sample from the magnetic rack. Add **700 µl** Buffer PE directly to the bead pellet. Incubate at **25°C** and **1400 rpm** for **1 min**.

Repeat

m) **Repeat** steps k and l for a total of 2 washes.

n) Centrifuge the tube briefly and place on the magnetic rack for **1 min** to allow bead capture. Remove and discard the supernatant. Take care not to disturb the bead pellet.

CRITICAL!

Leave the sample on the magnetic rack for the next step. Do not pipette water directly onto the beads.

The timing of the next step is extremely important. If a multi-channel pipette is not available, ensure that each tube has the exact same incubation time. Do not exceed 1 min.

o) Carefully add **700 µl** Nuclease-Free Water down the side of the tube **opposite** the magnetic pellet. Incubate for **exactly 1 min**. Promptly remove and discard the supernatant.

Repeat

p) **Repeat** step o for a total of 2 washes.

q) Remove the sample from the magnetic rack. Add **150 µl** Buffer AE directly to the bead pellet and incubate at **25°C** and **1400 rpm** for **3 min**.

r) Centrifuge the tube briefly and place on a magnetic rack for **1 min** to allow bead capture.

s) Using a **wide-bore** pipette tip, carefully transfer the supernatant containing purified gDNA to a new 1.5 ml low-bind screw-cap tube.

t) Store the extracted gDNA sample at **4°C** for up to **2 weeks** or at **–20°C** for up to **6 months** or proceed directly to GEM Generation & Barcoding.

Protocol Step 2

GEM Generation & Barcoding

Partition template HMW gDNA across millions of GEMs for barcoding

2

2. 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. USA Scientific, Eppendorf, and Thermo Fisher PCR 8-tube strips have also been validated. Substituting these materials can adversely affect performance.

Importance of DNA Loading Quantity

It is critical to quantify template HMW gDNA accurately to load the correct amount into the Sample Master Mix. Operating outside the recommended input amount can result in a reduction in application performance.

- Underloading the mass of genomic input material into the system will typically result in a higher PCR duplication rate and a reduction in variant-calling performance.
- Overloading the genomic input material will reduce the long-range information available for haplotype phasing and structural variant calling due to lower Linked-Reads per molecule.

High Sensitivity DNA Quantification

The Qubit® Fluorometer system is recommended for template gDNA quantification prior to creating the Sample Master Mix. Refer to the manufacturer's handbook for operation of the Fluorometer and the Qubit High Sensitivity (HS) protocol kits.

Best Practices – HMW gDNA Handling

Maintaining the integrity of HMW gDNA throughout GEM generation is important in achieving long haplotype phase blocks and the ability to call structural variants.

- Never vortex tubes containing HMW gDNA.
- Use wide-bore pipette tips for mixing HMW gDNA.
- Pipette slowly at all times to avoid shearing (3 sec down stroke, 3 sec up stroke for wide-bore pipette tips). If using narrow-bore pipette tips, increase the time for each stroke to 5 sec.
- For mixing, slowly draw >80% of the solution into the pipette tip then gently discharge at the solution surface 10 times.
- Use narrow-bore pipette tips for transferring HMW gDNA for accuracy.

Best Practices – Reagent Handling

Timely and proper handling of reagents are important for optimal performance.

- Ensure 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.
- Equilibrate the Genome Gel Bead Strip to room temperature for >30 min before use and store any unused Genome Gel Beads at –80°C. Avoid more than 10 freeze-thaw cycles at –80°C and never store at –20°C.

Best Practices – GEM Generation

Preserving the integrity of the HMW gDNA throughout GEM generation and proper handling of the Genome Chip are key to optimal performance:

- Gently mix HMW gDNA samples or Sample Master Mix containing HMW gDNA 10 times with wide-bore pipette tips before use.
- Minimize exposure of reagents, chips, and gaskets to sources of fibers such as reagent reservoirs, KimWipes, repeat-usage of flip-cap tubes, and the general laboratory environment.
- Start GEM generation immediately after Genome Chip loading.
- Unload GEMs immediately after the completion of GEM generation.
- If running more than 8 samples, maintain the capped tube strips with recovered GEMs on ice.
- Cover Partitioning Oil tubes and holders to minimize evaporation.

Prepare 50% Glycerol Solution if Processing Fewer than 8 Reactions

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 Genome Chip prior to running the Chromium™ Controller. See Practical Tips & Troubleshooting (Section 6) for information on purchasing or generating a 50% glycerol solution.

Additional Practical Tips & Troubleshooting

Further information can be found in Practical Tips & Troubleshooting (Section 6):

- Processing fewer than 8 reactions.
- Pipetting Gel Beads and GEMs.
- Assembling a Chromium Chip and a 10x™ Chip Holder.
- Reagent clogs during GEM generation.
- Chromium Controller errors.

Getting Started! – Genome v2 (16 rxns) Kit

Equilibrate to room temperature before use:

Item	Part Number	Storage Location
Qubit® HS standards	-	Manufacturer's recommendations
Genome Gel Beads *Equilibrate to room temperature 30 min before loading the Genome Chip*	220058	**–80°C**
Additive A *Vortex, verify no precipitate, centrifuge briefly*	220074	–20°C
Denaturing Agent *Vortex, centrifuge briefly*	220072	–20°C

50% glycerol solution:

If processing fewer than 8 reactions, see Practical Tips & Troubleshooting (Section 6) for information on purchasing or generating 50% glycerol solution

Place on ice:

Item	Part Number	Storage Location
Genome Reagent Mix *Thaw, vortex, centrifuge briefly*	220123	–20°C
Genome Enzyme Mix *Centrifuge briefly*	220122	–20°C
Chilled Metal Block	-	-

Obtain:

Item	Part Number	Storage Location
Partitioning Oil	220088	Ambient temperature
Genome Chip(s)	230028	Ambient temperature
10x™ Gasket(s)	370017	Ambient temperature
10x™ Chip Holder	330019	Ambient temperature
Qiagen Buffer EB	-	Manufacturer's recommendations

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

Getting Started! – HT v2 (96 rxns) Kit

Equilibrate to room temperature before use:

Item	Part Number	Storage Location
Qubit® HS standards	-	Manufacturer's recommendations
Genome Gel Bead Plate *Equilibrate to room temperature 30 min before loading the Genome Chip*	220102	**–80°C**
Additive A *Vortex, verify no precipitate, centrifuge briefly*	220093	–20°C
Denaturing Agent *Vortex, centrifuge briefly*	220072	–20°C

50% glycerol solution:

If processing fewer than 8 reactions, see Practical Tips & Troubleshooting (Section 6) for information on purchasing or generating 50% glycerol solution

Place on ice:

Item	Part Number	Storage Location
Genome Reagent Mix *Thaw, vortex, centrifuge briefly*	220114	–20°C
Genome Enzyme Mix *Centrifuge briefly*	220113	–20°C
Chilled Metal Block	-	-

Obtain:

Item	Part Number	Storage Location
Partitioning Oil	220088	Ambient temperature
Genome Chip(s)	230028	Ambient temperature
10x™ Gasket(s)	370017	Ambient temperature
10x™ Chip Holder	330019	Ambient temperature
Qiagen Buffer EB	-	Manufacturer's recommendations

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

GEM Generation & Barcoding

2.1. Input HMW gDNA Quantification

If extracted HMW gDNA is freshly thawed from frozen, gently mix 10 times with a wide-bore pipette tip before continuing.

NOTE

Calculating the volume of gDNA solutions needed depends on the amount of material available and ideal requirements for all steps from initial stock concentration, through quantification, to denaturing the gDNA. When initial sample volumes are limiting, lower volumes can be prepared.

a) Prepare sufficient Qubit® working solution for at least 4 Qubit readings per sample + 2 standards. See Manufacturer's recommendations for preparation and usage.

NOTE

If extracted gDNA stock solution concentration is already known, skip step b and proceed to to step c.

b) Quantitate **3 µl** of extracted gDNA solution (with a minimum of 2 replicates).

c) If the gDNA stock is **>20 ng/µl**, prepare an intermediate dilution of the extracted gDNA solution at **<20 ng/µl** in Buffer EB. Gently mix 10 times with a **wide-bore** pipette tip.

NOTE

Ideally prepare a total of 30 µl at 5-20 ng/µl. This will ensure enough material for two quantification replicates and preparation of sufficient volume at the final concentration.

d) Quantitate **3 µl** of the **<20 ng/µl** intermediate gDNA stock (with a minimum of 2 replicates) to verify the diluted concentration.

e) Dilute the gDNA solution to the **1 ng/µl** in Buffer EB in a PCR 8-tube strip. Gently mix 10 times with a multi-channel pipette and **wide-bore** pipette tips.

NOTE

Ideally prepare a total of 50 µl at 1 ng/µl. This will ensure enough material for two quantification replicates and 10 µl for the dentaturation step (and enough material for a second run, if needed).

f) Quantitate **3 µl** of the diluted gDNA solution from step e (with a minimum of 2 replicates) to verify the diluted concentration.

NOTE

If replicate concentration measurements differ by >15%, use a wide-bore pipette tip to gently mix the diluted sample 10 times and repeat the reading.

g) Verify recorded concentrations of the diluted gDNA solution are **0.8 – 1.2 ng/µl** before proceeding to Preparing GEM Reagent Mix. If recorded concentrations are out of range, repeat from step d.

2.2. Preparing Sample Master Mix

NOTE

If processing up to 3 Chromium™ Genome Chips, prepare all Sample Master Mix combined with Denatured gDNA (steps 2.2a – 2.2j) before proceeding with step 2.3.

a) Prepare the Sample Master Mix in a 1.5 ml or 5 ml tube on ice. Add reagents in the order shown below. Pipette mix thoroughly and centrifuge briefly. Maintain on ice.

NOTE

Volumes for 8 or 24 reactions are listed in all reagent tables and a designated excess.

To set up a different number of reactions (n), with a percent excess (e) multiply the indicated 1 reaction volumes by n + (n x e). For example, multiply by 4.4 to set up 4 reactions with 10% excess [4 + (4 x 0.1) = 4.4].

For v2 (16 rxns) Kit			
Sample Master Mix	**Part Number**	**1 rxn (µl)**	**8 rxns + 10% excess (µl)**
Genome Reagent Mix *Vortex, centrifuge briefly*	220123	89.5	788
Additive A *Vortex, centrifuge briefly*	220074	3	26
Genome Enzyme Mix *Centrifuge briefly*	220122	5	44
Total	-	**97.5**	**858**

For HT (96 rxns) Kit			
Sample Master Mix	**Part Number**	**1 rxn (µl)**	**24 rxns + 10% excess (µl)**
Genome Reagent Mix *Vortex, centrifuge briefly*	220114	89.5	2363
Additive A *Vortex, centrifuge briefly*	220093	3	79
Genome Enzyme Mix *Centrifuge briefly*	220113	5	132
Total	-	**97.5**	**2574**

b) Dispense **97.5 µl** Sample Master Mix per reaction into a PCR 8-tube strip and place the tube strip on a chilled metal block resting on ice.

c) Dispense **10 µl** Denaturing Agent into a **new** tube strip at room temperature.

d) Slowly transfer **10 µl** diluted gDNA from step 2.1 into the pre-aliquoted Denaturing Agent using a multi-channel pipette and **narrow-bore** pipette tips.

NOTE

If the volume of diluted gDNA available is <10 µl, adjust the volume of Denaturing Agent aliquots in step c to equal the volume of diluted gDNA available for transfer.

e) Gently mix the combined gDNA and Denaturing Agent 10 times with a multi-channel pipette and **wide-bore** pipette tips.

f) Incubate the combined gDNA and Denaturing Agent for **5 min** at **room temperature**.

g) Slowly add **2.5 µl** denatured gDNA to **97.5 µl** pre-dispensed Sample Master Mix (from step b) with a multi-channel pipette while on ice.

h) After all denatured gDNA samples are added, simultaneously and gently mix all samples 10 times using a multi-channel pipette set to **90 µl** with **wide-bore** pipette tips.

i) Briefly centrifuge the tube strip and return to the chilled block.

2.3. Loading the Genome Chip

a) Place a Genome 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 6) for tips on assembly.

CRITICAL!

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

b) If processing fewer than 8 samples per Genome Chip, first add the following volumes of 50% glycerol solution to each unused well:

i. **90 µl** in the row labeled 1

ii. **85 µl** in the row labeled 2

iii. **270 µl** in the row labeled 3

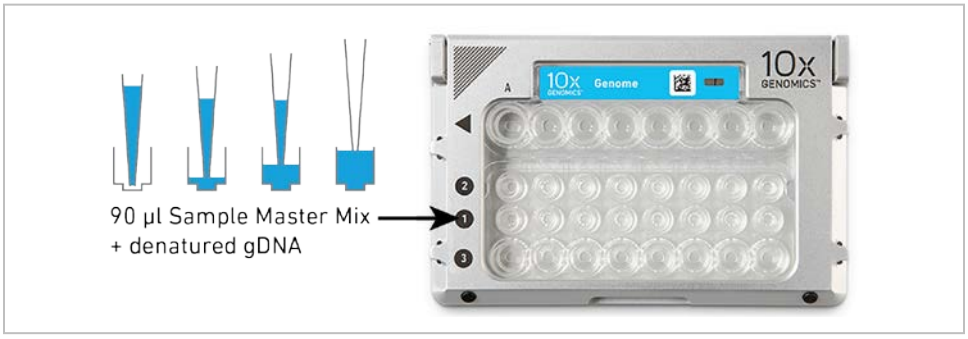
CRITICAL!

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.

c) Using a **narrow-bore** pipette tip, slowly transfer **90 µl** Sample Master Mix-denatured gDNA mixture **into the bottom of 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 Sample Master Mix-denatured gDNA mixture.

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 sample well, keeping the tip slightly submerged.



d) Gel Bead Preparation in Gel Bead Strips - **For v2 (16 rxns) Kit**

i. Snap the Genome Gel Bead Strip into a 10x™ Vortex Adapter. Vortex for **30 sec**.

ii. Remove the Genome 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 liquid levels look even.

iii. Carefully puncture the foil seal and slowly aspirate **85 µl** Genome Gel Beads.

d) Gel Bead Preparation in Gel Bead Plates - **For HT (96 rxns) Kit**

i. Centrifuge the Genome Gel Bead Plate at **300 rcf** for **30 sec**.

ii. Carefully puncture the foil seal and pipette mix **20 times**, taking care not to introduce any air bubbles, which would lead to foaming in the Gel Beads and insufficient Gel Bead recovery.

iii. Using the same pipette tips, slowly aspirate **85 µl** Genome Gel Beads.

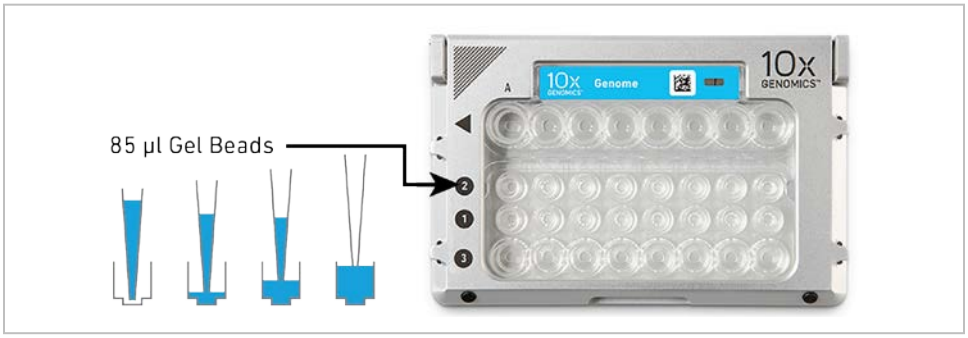
NOTE

Pipette Genome Gel Beads slowly as they have a viscosity similar to high-concentration glycerol. Only puncture the foil of a number of wells equal to the number of samples that will be processed.

Equipment to Prepare Gel Bead Strip

Equipment to Prepare Gel Bead Plates

e) Slowly dispense the Genome Gel Beads **into the bottom of 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. Raise the pipette tips at the same rate as the liquid level is rising in the Gel Bead wells, keeping the tip slightly submerged.

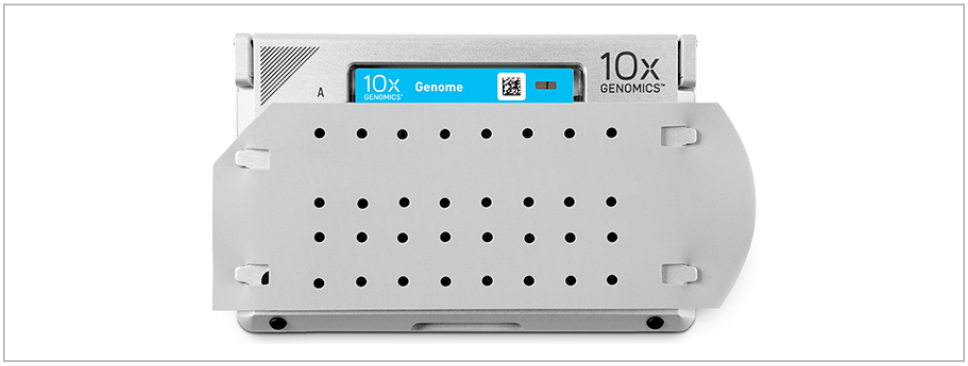


f) Pipette **270 µl** Partitioning Oil from a reagent reservoir into the wells in the **row labeled 3**. Do not add Partitioning Oil to any unused input wells that already contain 50% glycerol solution.

CRITICAL!

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

g) 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 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.



2.4. Running the Chromium™ 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 **Genome program** shows on screen and press the play button to begin the run.

d) At the completion of the run (~**20 min**), the Chromium Controller will chime. Proceed immediately to the next step.

	
Place the assembled Chip, 10x Chip Holder and 10x Gasket in the tray and press the button on the touchscreen to retract the tray	Confirm the Genome program shows on the screen and press the play button to start the run

2.5. Transferring GEMs

a) Maintain an emulsion-safe PCR 8-tube strip or PCR plate for GEM transfer on a chilled metal block resting on ice. (See Tips section for more information on emulsion-safe plastic consumables).

b) Press the eject button to eject the tray and remove the Genome 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 Genome Chip.

NOTE

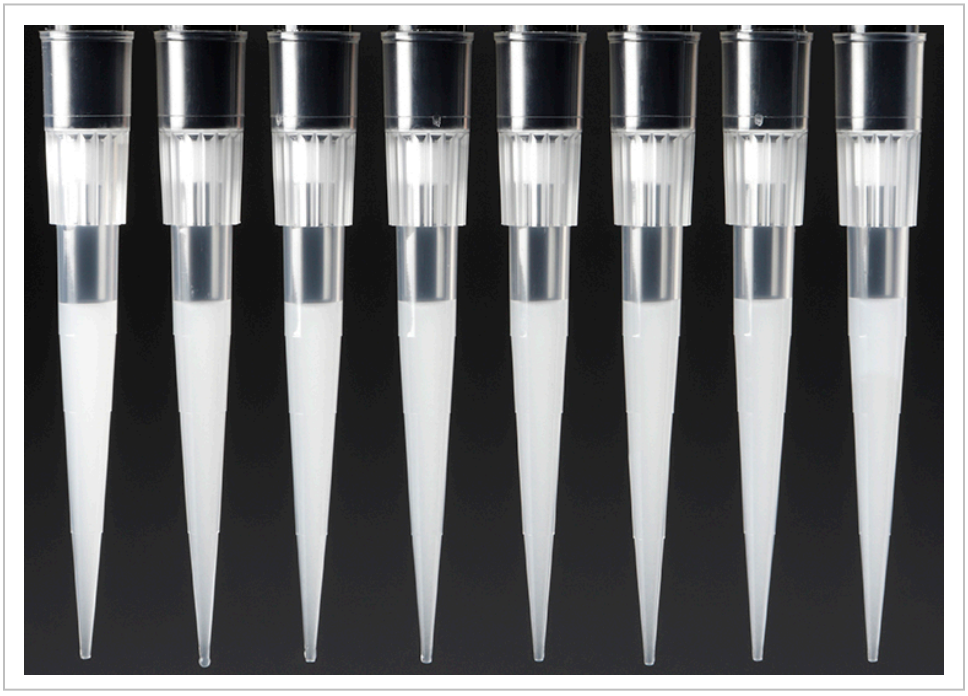
Abnormally high volume in any of the wells may indicate that a clog occurred during GEM generation.

e) Slowly aspirate **125 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.

NOTE

Pipette GEMs slowly as they have a high viscosity.

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.



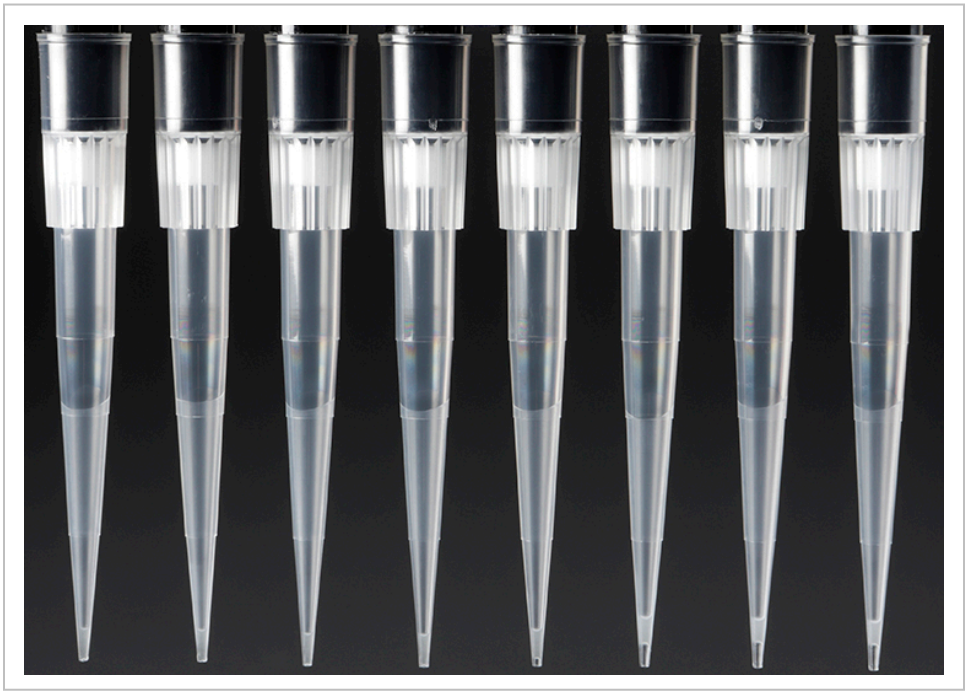
NOTE

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

g) Slowly dispense the GEMs into the tube strip or the plate on a chilled metal block resting on ice. Keep the tips above the liquid level to minimize GEMs lost on the outside of the tips.

NOTE

A white coating of GEMs may be left in the pipette tips as illustrated below. This is normal.



h) If multiple Genome Chips are run back-to-back, keep the tube strip or the plate containing recovered GEMs on ice. If using the plate, seal the plate wells containing GEMs with cap strips before proceeding to generate GEMs for the next set of samples.

i) Discard the used Genome Chip. Push the black sliders on the back of the 10x™ Chip Holder toward the middle to release the lock and close the lid.

NOTE

After workflow is completed, discard the remaining denatured gDNA (prepared in Step 2.2) and diluted gDNA solutions (prepared in Step 2.1) as DNA is not stable at these concentrations.

2.6. GEM Isothermal Incubation

a) If necessary, remove the cap strips from the PCR 8-tube strip or the PCR plate with recovered GEMs.

b) If using the plate, check that the Plate Sealer plate block is at room temperature. Seal the plate with pierceable foil heat seal at **185°C** for **6 sec** and promptly remove.

c) Load the tube strip or the plate into a thermal cycler that can accommodate 125 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
75°C	125 µl	~3 h 10 min
Step	**Temperature**	**Time**
1	30°C	3:00:00
2	65°C	10:00
3	4°C	Hold

STOP

d) Store in the PCR 8-tube strip or the PCR plate at **4°C** for up to **72 h** or at **–20°C** for up to **2 weeks** before proceeding to Post GEM Incubation Cleanup.

Protocol Step 3

Post GEM Incubation Cleanup & QC

Isolate and size DNA for library construction

3

3. Post GEM Incubation Cleanup & QC

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.

Best Practices – SPRIselect Reagent Ratio

Nucleic acid size selection using SPRIselect Reagent is extremely sensitive to the exact volumes of sample and SPRIselect Reagent: the SPRIselect Reagent ratio. Ensure that pipettes are properly calibrated and pipetting volumes are carefully followed for each SPRIselect Cleanup step.

Best Practices – Post GEM QC

Agilent Bioanalyzer is the recommended method for library QC after GEM cleanup to ensure successful library recovery, before proceeding with library construction.

Getting Started! – Genome v2 (16 rxns) Kit

Equilibrate to room temperature before use:

Item	Part Number	Storage Location
● Additive A *Vortex, verify no precipitate, centrifuge briefly*	220074	–20°C
DynaBeads® MyOne™ Silane beads *Vortex, centrifuge briefly*	-	Manufacturer's recommendations
Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations
Agilent Bioanalyzer High Sensitivity Kit	-	Manufacturer's recommendations

Obtain:

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

Thaw at 65°C:

Item	Part Number	Storage Location
● Buffer Sample Clean Up 1 *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.*	220020	–20°C

Prepare fresh 80% Ethanol (10 ml for 8 samples)

Getting Started! – HT v2 (96 rxns) Kit

Equilibrate to room temperature before use:

Item	Part Number	Storage Location
● Additive A *Vortex, verify no precipitate, centrifuge briefly*	220093	–20°C
DynaBeads® MyOne™ Silane beads *Vortex, centrifuge briefly*	-	Manufacturer's recommendations
Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations
Agilent Bioanalyzer High Sensitivity Kit	-	Manufacturer's recommendations

Obtain:

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

Thaw at 65°C:

Item	Part Number	Storage Location
○ Buffer Sample Clean Up 1 *Thaw at 65°C with agitation, verify no visible crystals, let cool to room temperature*	220094	–20°C

Prepare fresh 80% Ethanol (30 ml for 24 samples)

Post GEM Incubation Cleanup & QC

3.1. Post GEM Incubation Cleanup – Silane DynaBeads

a) At room temperature, add **125 µl** Recovery Agent to each tube or well containing post incubation GEMs. Pipette mix thoroughly.

b) Cap the tube strip and place in a 10x™ Vortex Clip. Vortex for **15 sec**.

c) Briefly centrifuge the tube strip. The resulting 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.

d) Slowly remove **135 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.

NOTE

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

e) Vortex DynaBeads MyOne Silane beads until fully resuspended. Prepare the DynaBeads Cleanup Mix by adding reagents in the order shown below. Vortex mix thoroughly and use immediately.

For v2 (16 rxns) Kit			
DynaBeads Cleanup Mix	**Part Number**	**1 rxn (µl)**	**8 rxns + 10% excess (µl)**
Buffer Sample Clean Up 1	220020	136	1197
DynaBeads MyOne Silane *Vortex, centrifuge briefly*	-	8	70
Additive A *Vortex, centrifuge briefly*	220074	6	53
Total	-	**150**	**1320**

For HT (96 rxns) Kit			
DynaBeads Cleanup Mix	**Part Number**	**1 rxn (µl)**	**24 rxns + 10% excess (µl)**
Buffer Sample Clean Up 1	220094	136	3590
DynaBeads MyOne Silane *Vortex, centrifuge briefly*	-	8	211
Additive A *Vortex, centrifuge briefly*	220093	6	159
Total	-	**150**	**3960**

f) Immediately add **150 µl** DynaBeads Cleanup Mix to each sample. Pipette mix thoroughly and incubate at **room temperature** for **10 min**.

CRITICAL! *Do not attempt to cap the tube strip as the liquid volume is high.*

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

For v2 (16 rxns) Kit			
Elution Solution I	**Part Number**	**1 rxn (µl)**	**8 rxns + 25% excess (µl)**
Buffer EB	-	89	890
10% Tween 20	-	1	10
Additive A *Vortex, centrifuge briefly*	220074	10	100
Total	-	**100**	**1000**

For HT (96 rxns) Kit			
Elution Solution I	**Part Number**	**1 rxn (µl)**	**24 rxns + 25% excess (µl)**
Buffer EB	-	89	2670
10% Tween 20	-	1	30
Additive A *Vortex, centrifuge briefly*	220093	10	300
Total	-	**100**	**3000**

h) After the 10 min incubation step is completed, place the tube strip into a 10x™ Magnetic Separator in the **High** position until the solution is clear (>2 min).

i) Carefully remove and discard the supernatant.

j) Add **250 µl** freshly prepared 80% ethanol to the pellet while on the magnet and stand for **30 sec**.

k) Carefully remove and discard the ethanol wash.

l) Add **200 µl** 80% ethanol to the pellet and stand for **30 sec.**

m) Carefully remove and discard the ethanol wash.

n) Centrifuge the tube strip briefly and return it to a 10x Magnetic Separator in the **Low** position until the solution is clear.

o) Remove and discard any remaining ethanol. Remove the tube strip from the magnet and immediately add **51 µl** Elution Solution I from a reservoir with a multi-channel pipette.

CRITICAL!

Silane Dynabeads dry very quickly at this step and may clump if Elution Solution I is not added immediately after removal of residual ethanol.

p) Incubate **30 sec** before resuspending the pellet in Elution Solution I. Pipette mix thoroughly until beads are fully resuspended.

NOTE

Silane Dynabeads can be difficult to resuspend due to residual reagents from the GEM reaction. Mix thoroughly with a pipette set to 40 µl to avoid introducing air bubbles.

q) Incubate the tube strip at room temperature for **5 min**.

r) Centrifuge the tube strip briefly and return it to a 10x™ Magnetic Separator in the **Low** position until the solution is clear.

s) Transfer **50 µl** of sample to a new tube strip.

3.2. Post GEM Incubation Cleanup – SPRIselect

NOTE

See Practical Tips & Troubleshooting (Section 6.6) for more information on calculating SPRIselect Reagent ratios.

a) Prepare Elution Solution II by adding appropriate volume of reagents in the order shown below. Vortex mix thoroughly and centrifuge briefly.

For v2 (16 rxns) Kit			
Elution Solution II	**Part Number**	**1 rxn (µl)**	**8 rxns + 25% excess (µl)**
Buffer EB	-	98	980
Additive A *Vortex, centrifuge briefly*	220074	2	20
Total	-	**100**	**1000**

For HT (96 rxns) Kit			
Elution Solution II	**Part Number**	**1 rxn (µl)**	**24 rxns + 25% excess (µl)**
Buffer EB	-	98	2490
Additive A *Vortex, centrifuge briefly*	220093	2	60
Total	-	**100**	**3000**

b) Vortex the SPRIselect Reagent until fully resuspended. Add **35 µl** SPRIselect Reagent (**0.7X**) to each sample in the tube strip. Pipette mix thoroughly and centrifuge briefly.

c) Incubate the tube strip at **room temperature** for **5 min**.

d) Place the tube strip in a 10x™ Magnetic Separator in the **High** position until the solution is clear (>2 min).

e) Carefully remove and discard the supernatant.

f) Add **125 µl** 80% ethanol to the pellet and stand for **30 sec**.

g) Carefully remove and discard the ethanol wash.

Repeat

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

i) Centrifuge the tube strip briefly and return it to a 10x Magnetic Separator in the **Low** position.

j) Remove and discard any remaining ethanol. Remove the tube strip from the 10x™ Magnetic Separator and immediately add **52.5 µl** Elution Solution II from a reservoir with a multi-channel pipette.

CRITICAL!

SPRIselect beads dry very quickly at this step and may clump if Elution Solution II is not added immediately after removal of residual ethanol.

k) Pipette mix thoroughly and incubate at room temperature for 5 min.

l) Centrifuge the tube strip briefly and place it in a 10x Magnetic Separator in the **Low** position until the solution is clear.

m) Transfer **52 µl** of sample to a new tube strip.

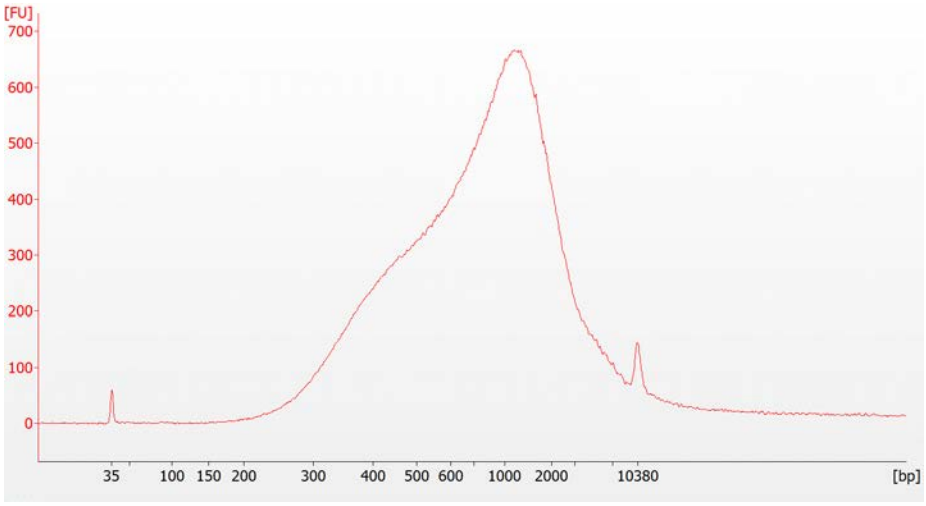
STOP

n) Store the samples in a tube strip at **4°C** for up to **72 h** or at **–20°C** for up to **2 weeks** before proceeding to Library Construction.

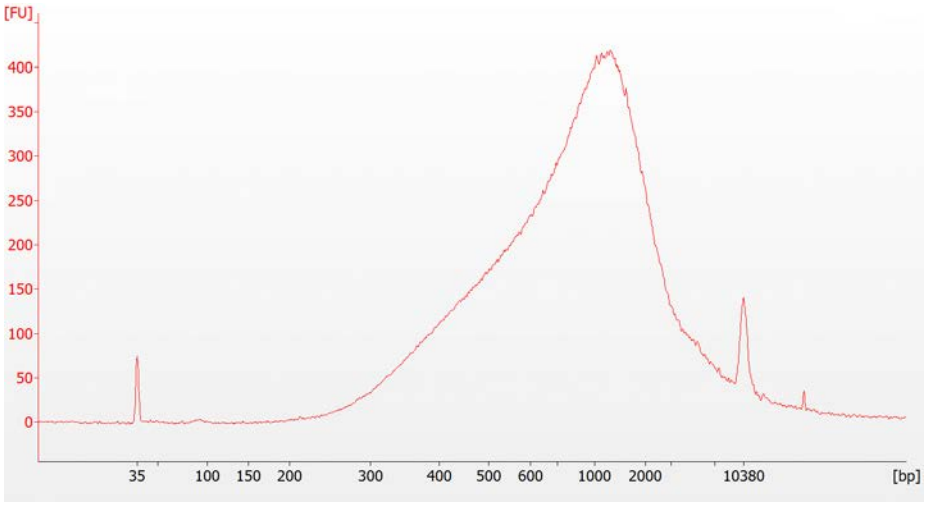
3.3. Post GEM QC

a) Run **1 µl** sample on the Agilent Bioanalyzer High Sensitivity DNA chip to determine yield and fragment size. Traces should resemble the overall shape of the sample electropherogram shown below. **Note the different x and y axis scales for each example.**

For v2 (16 rxns) Kit



For HT (96 rxns) Kit



Protocol Step 4

Library Construction

Insert P5 and P7 primers, Read 2, and Sample Index to prepare for sequencing

4. Library Construction

Tips

General

The final Genome Libraries contain the P5 and P7 primers used in Illumina® bridge amplification PCR. Read 1 sequence and the 10x™ Barcode are added to the molecules during the GEM incubation. P5 and P7 primers, Read 2, and Sample Index are added during library construction. These libraries are ready for sequencing after library construction.

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 – SPRIselect Reagent Ratio

Nucleic acid size selection using SPRIselect Reagent is extremely sensitive to the exact volumes of sample and SPRIselect Reagent: the SPRIselect Reagent ratio. Ensure that pipettes are properly calibrated and pipetting volumes are carefully followed for each SPRIselect Cleanup step.

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.

Best Practices – Post Library Construction QC

For post-library construction QC, Agilent Bioanalyzer, Agilent TapeStation, and Fragment Analyzer™ are suitable for determining fragment size and yield, before proceeding with library quantification and sequencer loading.

Getting Started! – Genome v2 (16 rxns) Kit

Equilibrate to room temperature before use:

Item	Part Number	Storage Location
End Repair and A-tailing Buffer *Vortex, verify no precipitate, centrifuge briefly*	220120	–20°C
Forward PCR Primer *Vortex, centrifuge briefly*	220124	–20°C
Adaptor Mix *Vortex, centrifuge briefly*	220026	–20°C
Ligation Buffer *Vortex, verify no clear pellet, centrifuge briefly*	220109	–20°C
Chromium™ i7 Sample Index Plate	220103	–20°C
Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations
Agilent Bioanalyzer DNA 1000 kit *If used for QC*	-	Manufacturer's recommendations
Agilent TapeStation D1000 ScreenTape and Reagents *If used for QC*	-	Manufacturer's recommendations

Obtain:

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

Place on ice:

Item	Part Number	Storage Location
End Repair and A-tailing Enzyme *Maintain on ice, centrifuge briefly before adding to End Repair and A-tailing Mix*	220121	–20°C
DNA Ligase *Maintain on ice, centrifuge briefly before adding to Adaptor Ligation Mix*	220110	–20°C
Amplification Master Mix *Maintain on ice, centrifuge briefly before adding to Sample Index PCR mix*	220125	–20°C
Kapa DNA Quantification Kit for Illumina® Platforms	-	Manufacturer's recommendations

Prepare fresh 80% Ethanol (10 ml for 8 samples)

Getting Started! – HT v2 (96 rxns) Kit

Equilibrate to room temperature before use:

Item	Part Number	Storage Location
End Repair and A-tailing Buffer *Vortex, verify no precipitate, centrifuge briefly*	220115	–20°C
Forward PCR Primer *Vortex, centrifuge briefly*	220101	–20°C
Adaptor Mix *Vortex, centrifuge briefly*	220099	–20°C
Ligation Buffer *Vortex, verify no clear pellet, centrifuge briefly*	220117	–20°C
Chromium™ i7 Sample Index Plate	220103	–20°C
Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations
Agilent Bioanalyzer DNA 1000 kit *If used for QC*	-	Manufacturer's recommendations
Agilent TapeStation D1000 ScreenTape and Reagents *If used for QC*	-	Manufacturer's recommendations

Obtain:

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

Place on ice:

Item	Part Number	Storage Location
End Repair and A-tailing Enzyme *Maintain on ice, centrifuge briefly before adding to End Repair and A-tailing Mix*	220116	–20°C
DNA Ligase *Maintain on ice, centrifuge briefly before adding to Adaptor Ligation Mix*	220118	–20°C
Amplification Master Mix *Maintain on ice, centrifuge briefly before adding to Sample Index PCR mix*	220119	–20°C
Kapa DNA Quantification Kit for Illumina® Platforms	-	Manufacturer's recommendations

Prepare fresh 80% Ethanol (30 ml for 24 samples)

Library Construction

4.1. End Repair & A-tailing

a) Vortex the End Repair and A-tailing Buffer. Verify there is no precipitate before proceeding.

b) Prepare End Repair and A-tailing Mix by adding the reagents in the order shown below. Pipette mix thoroughly and centrifuge briefly.

For v2 (16 rxns) Kit			
End Repair and A-tailing Mix	**Part Number**	**1 rxn (µl)**	**8 rxns + 10% excess (µl)**
Nuclease-Free Water	-	2.5	22
End Repair and A-tailing Buffer *Vortex, centrifuge briefly*	220120	7.5	66
End Repair and A-tailing Enzyme *Centrifuge briefly*	220121	15	132
Total	-	**25**	**220**

For HT (96 rxns) Kit			
End Repair and A-tailing Mix	**Part Number**	**1 rxn (µl)**	**24 rxns + 10% excess (µl)**
Nuclease-Free Water	-	2.5	66
End Repair and A-tailing Buffer *Vortex, centrifuge briefly*	220115	7.5	198
End Repair and A-tailing Enzyme *Centrifuge briefly*	220116	15	396
Total	-	**25**	**660**

c) Add **25 µl** End Repair and A-tailing Mix to each tube containing **50 µl** sample from Post GEM Incubation Cleanup. Pipette mix thoroughly and centrifuge briefly.

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

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

e) Proceed immediately to the next step.

4.2. Adaptor Ligation

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

For v2 (16 rxns) Kit			
Adaptor Ligation Mix	**Part Number**	**1 rxn (µl)**	**8 rxns + 10% excess (µl)**
Ligation Buffer *Vortex, centrifuge briefly*	220109	22	194
DNA Ligase *Vortex, centrifuge briefly*	221110	11	97
Adaptor Mix *Vortex, centrifuge briefly*	220026	2.5	22
Total	-	**35.5**	**313**

For HT (96 rxns) Kit			
Adaptor Ligation Mix	**Part Number**	**1 rxn (µl)**	**24 rxns + 10% excess (µl)**
Ligation Buffer *Vortex, centrifuge briefly*	220117	22	580
DNA Ligase *Vortex, centrifuge briefly*	220118	11	290
Adaptor Mix *Vortex, centrifuge briefly*	220099	2.5	66
Total	-	**35.5**	**936**

b) Add **35.5 µl** Adaptor Ligation Mix to each tube containing **75 µl** of sample from the End Repair and A-tailing step. Pipette mix thoroughly 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	15:00

d) Proceed **immediately** to the next step.

4.3. Post Ligation Cleanup – SPRIselect

a) Vortex the SPRIselect Reagent until fully resuspended. Add **88 µl** SPRIselect Reagent (**0.8X**) to each sample in the tube strip. Pipette mix thoroughly.

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 (>2 min).

d) Carefully remove and discard the supernatant.

e) Add **250 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. Remove the tube strip from the 10x Magnetic Separator and immediately add **40.5 µl** Buffer EB.

j) Pipette mix thoroughly and incubate at room temperature for **5 min**.

k) Centrifuge the tube strip briefly and return it to a 10x Magnetic Separator in the **Low** position until the solution is clear.

l) Transfer **40 µl** of sample to a new tube strip and proceed immediately to the next step.

4.4. 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 the Sample Index PCR Mix by adding the reagents in the order shown below. Pipette mix thoroughly and centrifuge briefly.

For v2 (16 rxns) Kit			
Sample Index PCR Mix	**Part Number**	**1 rxn (µl)**	**8 rxns + 10% excess (µl)**
Amplification Master Mix *Centrifuge briefly*	220125	50	440
Forward PCR Primer *Vortex, centrifuge briefly*	220124	5	44
Total	-	**55**	**484**

For HT (96 rxns) Kit			
Sample Index PCR Mix	**Part Number**	**1 rxn (µl)**	**24 rxns + 10% excess (µl)**
Amplification Master Mix *Centrifuge briefly*	220119	50	1320
Forward PCR Primer *Centrifuge briefly*	220101	5	132
Total	-	**55**	**1452**

b) Add **55 µl** Sample Index PCR Mix to each tube containing **40 µl** purified Post Ligation sample.

c) Add **5 µl** of different Chromium i7 Sample Index to each well and record their assignment. Pipette mix thoroughly and centrifuge briefly.

d) Index the library DNA in a thermal cycler for a total of 10 cycles.

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~30 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, 9X (for 10 cycles in total)	
6	72°C	1:00
7	4°C	Hold

STOP

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

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

NOTE

See Practical Tips & Troubleshooting (Section 6.6) for more information on calculating SPRIselect Reagent ratios.

a) Transfer **96 µl** of the indexed PCR samples to a new tube strip. If the sample is less than 96 µl, bring the total volume to 96 µl with Buffer EB.

b) Vortex the SPRIselect Reagent until fully resuspended. Add **48 µl** SPRIselect Reagent (**0.5X**) to each 96 µl sample in the tube strip. Pipette mix thoroughly.

c) Incubate the tube strip at room temperature for **5 min**.

d) Place the tube strip in a 10x™ Magnetic Separator in the **High** position until the solution is clear (>2 min).

CRITICAL!

DO NOT discard supernatant.

e) Transfer **135 µl** supernatant to a new tube strip and discard previous tube strip.

f) Vortex the SPRIselect Reagent until fully resuspended. Add **18 µl** SPRIselect Reagent (**0.7X** final) to the supernatant in the new tube strip. Pipette mix thoroughly.

g) Incubate the tube strip at room temperature for **5 min**.

h) Place the tube strip in a 10x Magnetic Separator in the **High** position until the solution is clear.

i) Carefully remove and discard the supernatant.

j) Add **200 µl** 80% ethanol to the pellet and stand for **30 sec**.

k) Carefully remove and discard the ethanol wash.

Repeat

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

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. Remove the tube strip from the 10x Magnetic Separator and immediately add **20.5 µl** Buffer EB.

o) Pipette mix thoroughly and incubate at room temperature for **5 min**.

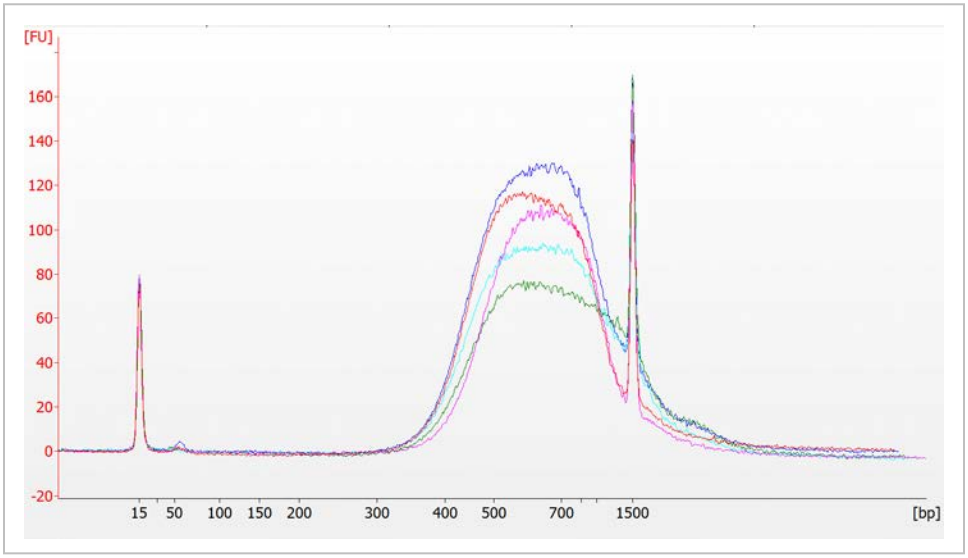
p) Centrifuge the tube strip briefly and return it to a 10x Magnetic Separator in the **Low** position until the solution is clear.

q) Transfer **20 µl** of sample to a new tube strip.

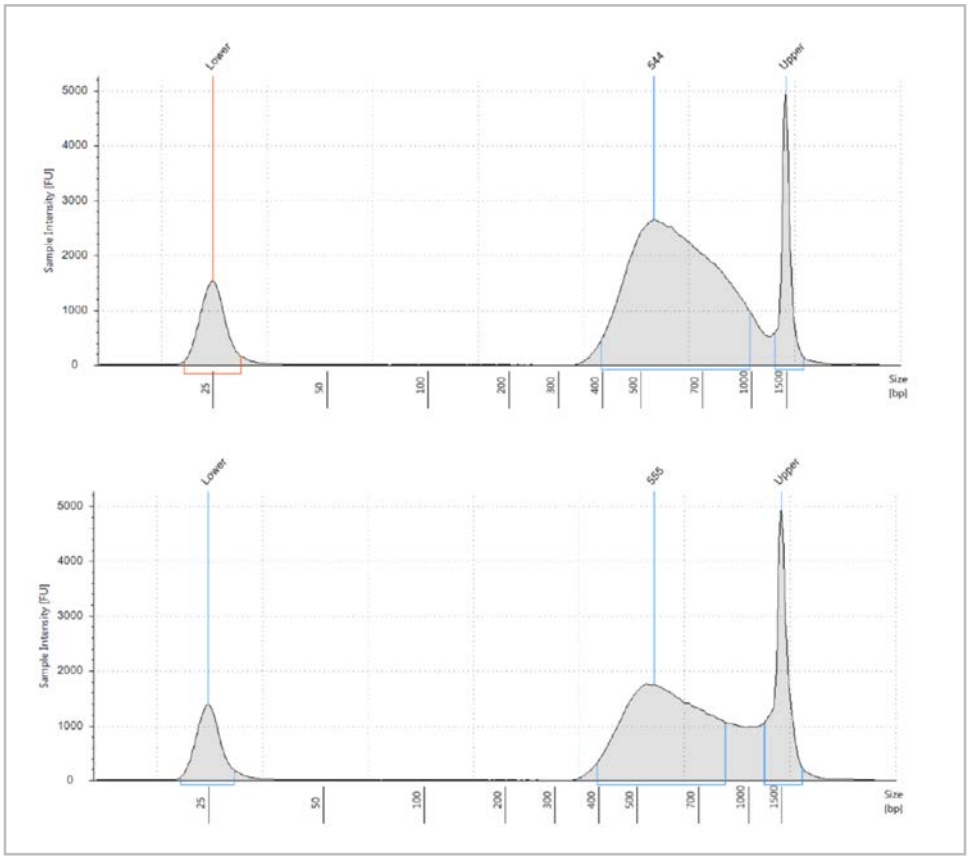
r) Store the tube strip at **4°C** for up to **72 h** or at **–20°C** for **long-term** storage.

4.6. Post Library Construction QC

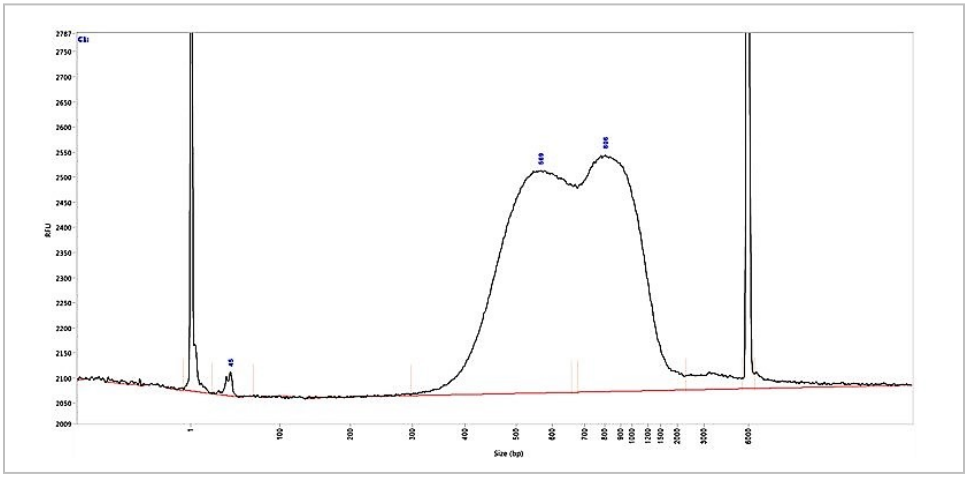
a) **EITHER** Run **1 µl** sample on the Agilent Bioanalyzer DNA 1000 chip to determine fragment size. Verify that a library has been generated by looking for a distribution similar to one illustrated below.



b) **OR** Run **1 µl** sample on the Agilent TapeStation D1000 ScreenTape to determine fragment size. Verify that a library has been generated by looking for a distribution similar to those illustrated below.



c) **OR** Run **2 µl** sample at 1:40 dilution on the Fragment Analyzer™ Automated CE system. Verify that a library has been generated by looking for a distribution similar to the one illustrated below.



4.7. 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 Genome library is required to bring the library within the dynamic range of the assay.

a) Thaw Kapa DNA Quantification Kit for Illumina® platforms.

b) Dilute **1 µl** of sample with deionized water to appropriate dilutions that fall within the linear detection range of the Kapa DNA Quantification Kit. (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	**1 rxn (µl)**
SYBR Fast Master Mix + Primer	12
Water	4
Total	**16**

d) Dispense **16 µl** of Quantification Master Mix for sample dilutions and DNA Standards into a PCR 8-tube strip or a 96-well PCR Plate.

e) Add **4 µl** of sample dilutions and **4 µl** DNA Standards to appropriate wells. Centrifuge the PCR 8-tube strip or the 96-well 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-based quantification. For library quantification for sequencer clustering, determine the concentration using a fixed insert size of 550 bp.

Protocol Step 5

Sequencing

Sequencing prepared libraries

5

5. Sequencing Libraries

The Chromium™ Genome Solution produces Illumina® sequencing-ready libraries. This section describes the steps required to ensure the successful sequencing of Genome libraries to deliver the full value of the Chromium Genome Solution.

Genome libraries comprise standard Illumina paired-end constructs which begin with P5 and end with P7. The 16 bp 10x™ Barcode is 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.

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

5.1. Sequencing Depth Recommendations

The sequencing performance of Genome libraries is driven by both sequencing coverage and total number of reads. The following are recommended when performing genome sequencing.

Specifications	Recommended Coverage (Human)
Gigabases of Sequence	128
Reads Passing Filter	850 Million (425 Million read pairs)
Targeted Deduped Depth	>30x

5.2. Sequencing Run Parameters

a) Genome libraries use standard Illumina® sequencing primers for both sequencing and index reads, and require no custom primers.

b) Genome libraries are run using paired-end sequencing with single indexing. The supported number of cycles for each read is shown below.

Sequencing Read	Recommended Number of Cycles
Read 1	150 cycles
i7 index	8 cycles
i5 index	0 cycles
Read 2	150 cycles

5.3. Sample Indices

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

Practical Tips & Troubleshooting

Processing Fewer than 8 Reactions

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

Pipetting Gel Bead Strips & GEMs

Pipetting Gel Bead Plates & GEMs

50% Glycerol Solution

SPRIselect Cleanups & Double Sided Size Selections

Reagent Clogs during GEM Generation

Chromium™ Controller Errors

Glossary of Terms

6

6. Practical Tips & Troubleshooting

6.1. 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 10 freeze-thaw cycles.

c) Never store Gel Beads at –20°C.

d) Reagent volumes should be calculated with a 10% excess of 1X values quoted in the protocol. E.g., for 3 samples, multiply the 1X 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) Minimize the number of freeze-thaw cycles and time that the reagents are opened to the lab environment to minimize reagent clogs and ensure optimal system performance. Avoid more than 10 freeze-thaw cycles for Gel Bead and non-Gel Bead reagents.

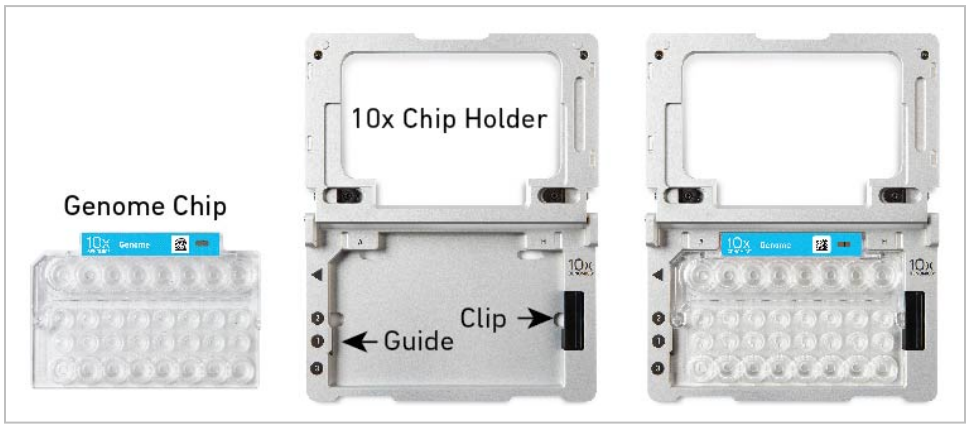
6.2. Assembling a Chip, 10x™ Chip Holder & 10x™ Gasket

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.

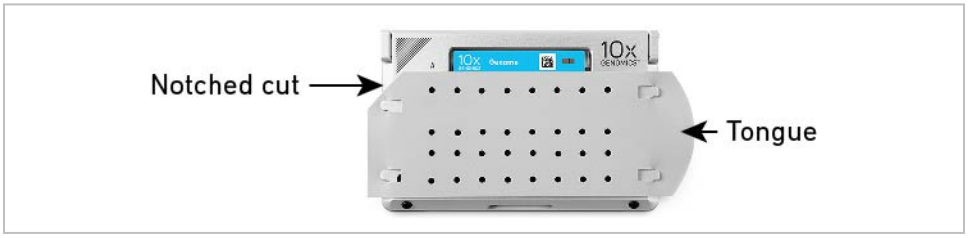
b) Depress the right-hand side of the Chromium Chip until the spring-loaded clip engages the Chromium Chip.



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.

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 with the notched cut at the top left corner, 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 Genome Chip

6.3. Pipetting Gel Bead Strips & GEMs – v2 (16 rxns) Kit

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 Genome Gel Bead recovery. Confirm that there are no bubbles at the bottom of the tube.

b) If processing fewer than 8 samples, only puncture sufficient wells in the Genome Gel Bead Strip just prior to loading the Genome Chip.

c) 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.

d) 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.

e) 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.

Click back to Loading the Genome Chip

6.4. Pipetting Gel Bead Plates & GEMs – HT (96 rxns) Kit

a) Centrifuge the Genome Gel Bead Plate at **300 rcf** for **30 sec**.

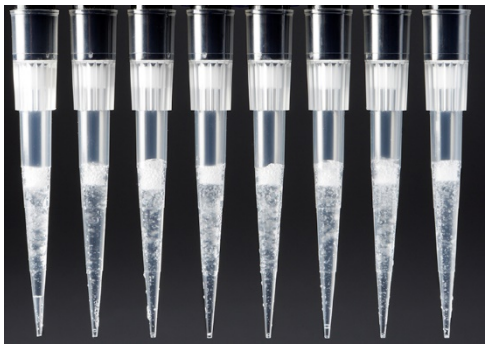
b) Carefully puncture sufficient wells in the Genome Gel Bead Plate just prior to pipette mixing and Chromium™ chip loading.

c) Best practices for pipette mixing the Gel Beads from the Gel Bead Plate include the following:

i. Set a pipette to 50 µl (80% of the volume being pipetted) and, without engaging the plunger, puncture the foil seal on the Gel Bead Plate. 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. Pipette mix the Gel Beads mix **20 times**, taking care not to introduce any air bubbles, which will lead to foaming in the Gel Beads and insufficient Gel Bead recovery.

Normal Gel Bead mixing and recovery	Foaming in the Gel Beads, which leads to insufficient Gel Bead recovery
	

iv. At the last mixing step, carefully dispense all the Gel Beads back into the wells of the plate. Reset the pipette volume to the Gel Bead dispense volume and carefully aspirate the Gel Beads using the same pipette tips.

d) 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.

e) 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.

Click back to Loading the Genome Chip

6.5. 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 Genome Chip prior to running the Chromium 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.

6.6. SPRIselect Cleanups & Double Sided Size Selections

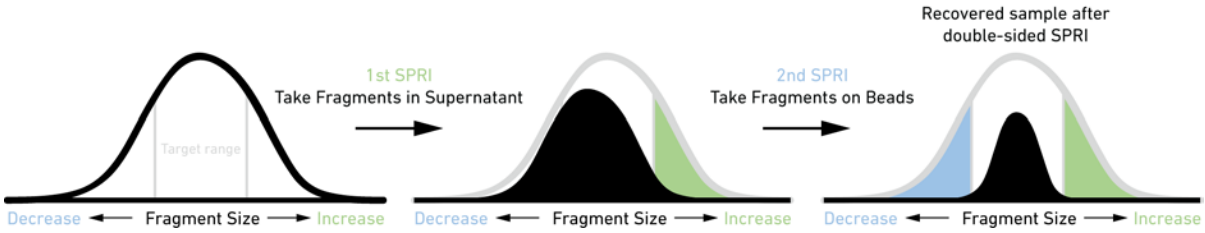
Solid Phase Reversible Immobilization (SPRI) beads selectively bind DNA fragments according to the ratio of SPRIselect Reagent (beads) to DNA solution. SPRIselect Reagent:DNA sample ratios indicated throughout the Protocol in SPRIselect Reagent steps are calculated as follows.

Example from Section 3.2 Post GEM incubation Cleanup

Step 3.2b: Add **35 µl** SPRIselect Reagent (**0.7X**) to each sample in the tube strip and pipette mix 15 times (pipette set to 150 µl).

$$\text{Ratio} = \frac{\text{Volume of SPRIselect Reagent added to the sample}}{\text{Volume of post GEM Incubation Cleanup-Silane DynaBead}} = \frac{35\ \mu l}{50\ \mu l} = \mathbf{0.7X}$$

Schematic Overview 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). Consult Technical Note SPRIselect: DNA Ratios Affect the Size Range of Library Fragments (Document CG000061) for more information on the use of SPRIselect Reagents.

Example from Section 4.5 Post Sample Index Double Sided Size Selection – SPRI Select

Step 4.5b: Add **48 µl** SPRIselect Reagent (**0.5X**) to each sample in the tube strip and pipette mix 15 times (pipette set to 75 µl).

$$\text{Ratio} = \frac{\text{Volume of SPRIselect Reagent added to the sample}}{\text{Volume of Indexed PCR sample (from Sample Index PCR)}} = \frac{48\ \mu l}{96\ \mu l} = \mathbf{0.5X}$$

Continued Example from Section 4.5 Post Sample Index Double Sided Size Selection – SPRI Select

Step 4.5f: Add **18 µl** SPRIselect Reagent (**0.7X** final) to each sample in the tube strip and pipette mix 15 times (pipette set to 75 µl).

$$\text{Ratio} = \frac{\textbf{Total}\ \text{volume of SPRIselect Reagent added to the sample (steps 4.5b + 4.5f)}}{\textbf{Original}\ \text{volume of Indexed PCR sample (from Sample Index PCR)}} = \frac{48\ \mu l + 18\ \mu l}{96\ \mu l} = \mathbf{0.7X}$$

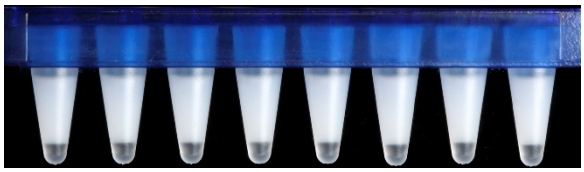
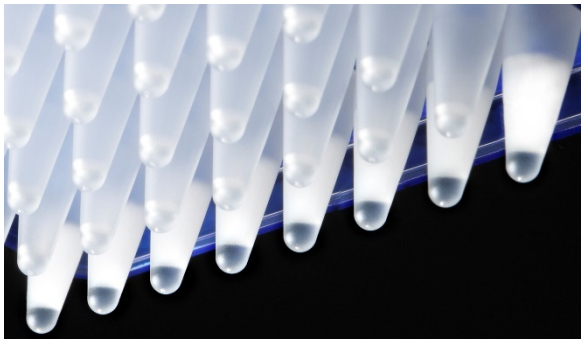
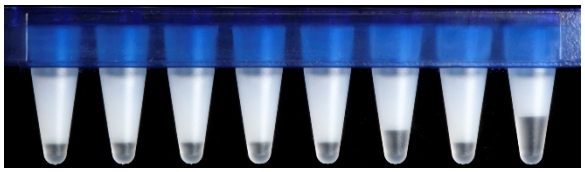
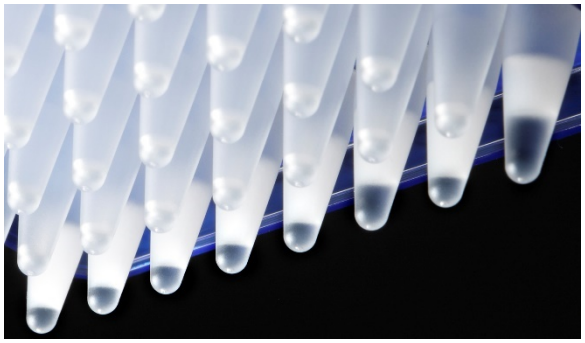
6.7. Reagent Clogs during GEM Generation

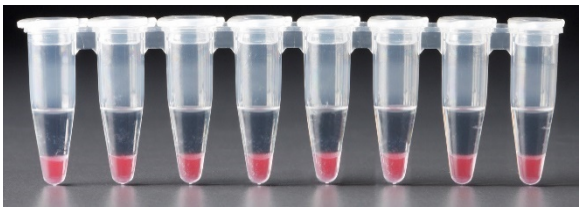
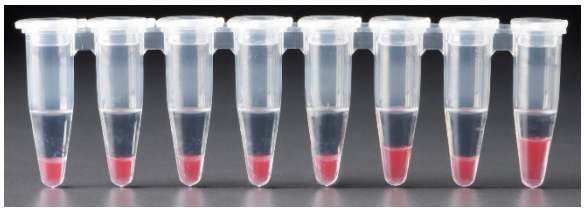
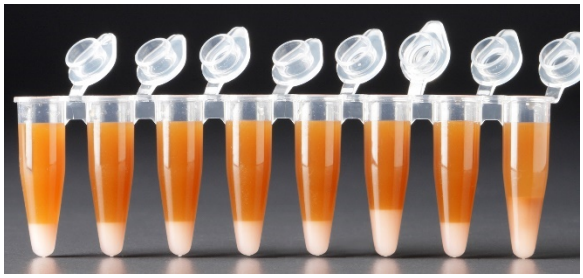
GEM reagents are manufactured in a cleanroom environment to minimize the level of particles and fibers that could clog microfluidic channels during GEM generation and therefore reduce technical performance.

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. Continue processing the remaining samples.

Normal Operations	Reagent Clogs
 A B C D E F G H **After aspirating the GEMs from the Chromium™ Genome Chip Recovery Wells:** All liquid levels are similar in volume and opacity. There is also no air in the pipette tips.	 A B C D E F G H **After aspirating the GEMs from the Chromium Genome Chip Recovery Wells:** Pipette tip F indicates a partial reagent clog has occurred in this channel. There is excess Partitioning Oil (clear) in the pipette tip. Pipette tip H indicates a major reagent clog has occurred in this channel. There is excess Partitioning Oil (clear) in the pipette tip. Pipette tips A – E and G are normal.

Normal Operations	Reagent Clogs
 A B C D E F G H  A B C D E F G H **After dispensing the GEMs into a PCR plate:** All liquid levels are similar in volume and opacity. Note that a thin layer of Partitioning Oil (clear) will naturally separate after GEMs are allowed to settle for >5 min.	 A B C D E F G H  A B C D E F G H **After dispensing the GEMs into a PCR plate:** Well F indicates a partial reagent clog has occurred in this channel. There is excess Partitioning Oil (clear) in the bottom of the well when compared to normal channels A – E and G. Well H indicates a major reagent clog has occurred in this channel. There is excess Partitioning Oil (clear) in the bottom of the well when compared to normal channels A – E and G.
 A B C D E F G H **After transfer of the GEMs + Recovery Agent to a tube strip:** All liquid levels are similar in the aqueous sample volume (clear) and Recovery Agent/Partitioning Oil (pink).	 A B C D E F G H **After transfer of the GEMs + Recovery Agent to a tube strip:** Tube F indicates a partial reagent clog has occurred in this channel. There is a decreased volume of aqueous sample (clear) when compared to normal channels A – E and G. Tube H indicates a major reagent clog has occurred in this channel. There is a decreased volume of aqueous sample (clear) when compared to normal channels A – E and G.

Normal Operations	Reagent Clogs
 A B C D E F G H **After removing the designated volume of Recovery Agent/Partitioning Oil:** All liquid volumes are similar in the aqueous sample volume (clear) and residual Recovery Agent/Partitioning Oil (pink).	 A B C D E F G H **After removing the designated volume of Recovery Agent/Partitioning Oil:** Tube F indicates a partial 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 A – E and G. Tube H indicates a major 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 A – E and G.
 A B C D E F G H **After addition of DynaBeads Cleanup Mix:** All liquid volumes are similar after addition of the DynaBeads Cleanup Mix.	 A B C D E F G H **After addition of DynaBeads Cleanup Mix:** Tube F indicates a partial 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 A – E and G. Tube H indicates a major 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 A – E and G.

6.8. Chromium™ Controller Errors

If the Chromium 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, 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 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

6.9. Glossary of Terms

10x™ Barcode

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

Chromium™ Genome Chip

The Chromium Genome Chip is a microfluidic chip specifically designed to run the Genome Protocol in the Chromium Controller. The Genome Chip is indicated by a blue label at the top of the 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 or 96-reaction Gel Bead Plates.

GEM

GEM is an abbreviation of Gel Bead-In-EMulsion. In the Genome Chip, a library of Genome Gel Beads is combined with an optimal amount of template HMW gDNA and a Reagent Master Mix to create reaction volumes, on the order of 100 picoliters, partitioned by oil.

GemCode™ Technology

The GemCode Technology is the microfluidic chip-based technology that partitions a high molecular weight genomic DNA sample across up to millions of GEMs. After reaction incubation, the fragments produced in each GEM contain a 10x Barcode that identify the fragments from having originated from the same input HMW gDNA molecule.

High Molecular Weight Genomic DNA

Inputting high molecular weight (HMW) genomic DNA (gDNA) into the Chromium Genome workflow is essential to optimal results by maximizing Linked-Reads per molecule, and optimizing the consequent reconstruction of previously inaccessible regions.

Linked-Reads

Linked-Reads are a novel data type generated by GemCode Technology. Linked-Reads are short read sequencing reads that contain a unique 10x Barcode, which is used to link individual short reads originating from the same single HMW gDNA input molecule (typically 50 – 100 kb). The input HMW gDNA sample is partitioned into individual GEMs, such that a single GEM is unlikely to contain more than one molecule from the same genomic locus. Linked-Reads per molecule is the number of short read sequencing reads with the same 10x Barcode that map to the same template HMW gDNA molecule.

Long-range Information

Long-range information is defined as the discovery, identification, and/or characterization of genome features up to hundreds of kilobases in size. GemCode Technology is able to address long-range information using a short read sequencer by generating Linked-Reads from HMW gDNA molecules, thus preserving the original structure of the underlying genome.