Exome Demonstrated Protocol

Application of the Chromium™ Genome Reagent Kits v2 for Exome Assays

10x GENOMICS®

10xGenomics.com

Notices

Manual Part Number

CG000059 Rev C

Legal Notices

Customer Information and Feedback

For technical information or advice, please contact our Customer Technical Support Division online at any time.

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Chromium™ Genome Reagent Kits v2

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 for Exome Application, 16 rxns PN-1000017			
Product	Description	#	Part Number
Chromium™ Genome Library Kit v2, 16 rxns (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, 16 rxns (store at –80°C)			120214
	Chromium™ Genome Gel Beads	2	220058
Chromium™ Post Capture Amplification Kit, 16 rxns (store at –20°C)			1000007
	Amplification Master Mix	1	220129
	Post Capture PCR Primers	1	220037

Chromium™ Genome HT 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 HT Library & Gel Bead Kit for Exome Application, 96 rxns PN-1000018			
Product	Description	#	Part Number
Chromium™ Genome HT Library Kit v2, 96 rxns (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, 96 rxns (store at –80°C)			120247
	Chromium™ Genome Gel Bead Plate (96 rxn)	1	220102
Chromium™ Post Capture Amplification Kit, 16 rxns (store at –20°C)*			1000007
	Amplification Master Mix	1	220129
	Post Capture PCR Primers	1	220037

* PN-1000018 contains 6 x PN-1000007.

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.*

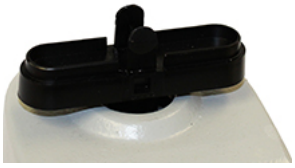
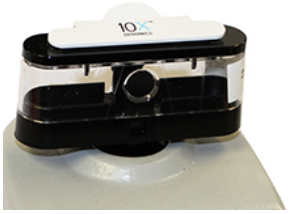
Chromium™ Genome Chip Kit, 48 rxns PN-120257			
Product	Description	#	Part Number
Chromium™ Genome Chip Kit v2, 48 rxns (store at ambient temperature)			120257
	Genome Chips	1	230028
	Gaskets	1	370017
	Partitioning Oil	4	220088
	Recovery Agent	6	220016

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

The Chromium™ Post Capture Amplification Kit is also available separately, as outlined below, for use in combination with the Chromium™ Genome Library & Gel Bead Kit v2, 16 rxns PN-120258 or the Chromium™ Genome HT Library & Gel Bead Kit, 96 rxns PN-120261.

Chromium™ Post Capture Amplification Kit, 16 rxns PN-1000007			
Product	Description	#	Part Number
Chromium™ Post Capture Amplification Kit, 16 rxns (store at –20°C)			1000007
	Amplification Master Mix	1	220129
	Post Capture PCR Primers	1	220037

Chromium™ Accessories

Product	Description	Part Number
10x™ Vortex Adapter	The 10x Vortex Adapter attaches to the top of a standard laboratory vortexer 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. Eppendorf twin.tec® brand PCR plates are highly recommended to ensure stability of GEM emulsions, and the specific model should be selected based on compatibility with thermal cycler in use. USA Scientific, Eppendorf and Thermo Fisher PCR 8-tube strips have also been validated. Substituting materials may adversely affect system performance. Either Bioanalyzer or TapeStation are needed for DNA quantification and quality control. Wide-bore tips are required for HMW gDNA handling.

Supplier	Description	Part Number (US)
Plastics		
Eppendorf	twin.tec® 96-Well PCR Plate Semi-skirted *Choose specific plate based on thermal cycler compatibility*	951020362
	twin.tec® 96-Well PCR Plate Divisible, Unskirted	0030133374
	twin.tec® 96-Well PCR Plate Unskirted	0030133390
	DNA LoBind Tubes, 1.5 ml	022431021
	DNA LoBind Tubes, 2.0 ml	022431048
	DNA LoBind Tubes, 5.0 ml	0030108310
	PCR Tubes 0.2 ml 8-tube strips *(alternate to USA Scientific product)*	951010022
Bio-Rad	Pierceable Foil Heat Seal	1814040
	Hard-Shell Low-Profile Thin-Wall 96-Well Skirted PCR Plates	HSP9665
USA Scientific	TempAssure PCR 8-tube strip *(alternate to Eppendorf product)*	1402-4700
Kits & Reagents		
Qiagen	Buffer EB	19086
Thermo Fisher Scientific	DynaBeads® MyOne™ Silane	37002D
	DynaBeads® MyOne™ Streptavidin T1	65604D
	Nuclease-Free Water	AM9937
Sigma	Ethanol, Pure (200 Proof, anhydrous)	459836-500ML
Beckman Coulter	SPRIselect Reagent Kit	B23318
Bio-Rad	10% Tween 20	1662404
Ricca Chemical Company	Glycerin (glycerol), 50% (v/v) Aqueous Solution	3290-32
Integrated DNA Technologies	xGen® Universal Blocking Oligo – TS-p5	1016184
	xGen® Universal Blocking Oligo – TS-p7(8nt)	1016188

Table continued on next page

Additional Kits, Reagents & Equipment

Table continued from previous page

Supplier	Description	Part Number (US)
Equipment		
Rainin	Tips LTS 200UL Filter RT-L200FLR	17007961
	Tips LTS W-O 200UL Fltr RT-L200WFLR	17014294
	Pipet-Lite Multi Pipette L8-50XLS+	17013804
	Pipet-Lite Multi Pipette L8-200XLS+	17013805
Bio-Rad	PX1™ PCR Plate Sealer	1814000
VWR	Vortex Mixer	10153-838
	Divided Polystyrene Reservoirs	41428-958
Covaris	M220 Focused Ultrasonicator™	500295
	M220 Holder microTUBE	500301
	microTUBE AFA Fiber Screw-Cap 6 x 16 mm	520096
Quantification & Quality Control		
Thermo Fisher Scientific	Qubit® 3.0 Fluorometer	Q33216
	Qubit® dsDNA HS Assay Kit	Q32854
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
KAPA Biosystems	Illumina® Library Quantification Kit	KK4824

Not all of these instruments and reagents are required.

Choose between Bioanalyzer and TapeStation 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.

Supplier	Description	Part Number (US)
Plastics		
Eppendorf	PCR Tubes 0.2 ml 8-tube strips *(alternate to USA Scientific product)*	0030124286
Thermo Fisher Scientific	MicroAmp® 8-Tube Strip, 0.2 ml *(alternate to USA Scientific product)*	N8010580
	MicroAmp® 8 -Cap Strip, clear	N8010535
Bio-Rad	Optical Flat 8-Cap Strips	TCS0803
	Microseal 'B' Adhesive Seals	MSB1001
Equipment		
Eppendorf	ThermoMixer C® *(alternate to USA Scientific product)*	5382000015
	SmartBlock 1.5 ml, Thermoblock for 24 Reaction Vessels (alternate *to USA Scientific product)*	5360000038
	SmartBlock™ PCR 96, thermoblock for PCR plates 96 *(alternate to USA Scientific product)*	5306000006
USA Scientific	Thermal-Lok 1-Position Dry Heat Bath *(alternate to Eppendorf product)*	2510-1101
	24-place 1.5/2.0 ml Thermal-Lok dry bath block *(alternate to Eppendorf product)*	2520-0000
Rainin	Tips LTS 20UL Filter RT-L10FLR	17007957
	Tips LTS 1ML Filter RT-L1000FLR	17007954
	Tips LTS W-O 1MLUL Fltr RT-L1000WFLR	17014297
	Pipet-Lite LTS Pipette L-2XLS+	17014393
	Pipet-Lite LTS Pipette L-20XLS+	17014392
	Pipet-Lite LTS Pipette L-10XLS+	17014388
	Pipet-Lite LTS Pipette L-20XLS+	17014392
	Pipet-Lite LTS Pipette L-100XLS+	17014384
	Pipet-Lite LTS Pipette L-200XLS+	17014391
	Pipet-Lite LTS Pipette L-1000XLS+	17014382
	Pipet-Lite Multi Pipette L8-10XLS+	17013802
	Pipet-Lite Multi Pipette L8-20XLS+	17013803
-	qPCR instrument and compatible consumables	

Recommended Thermal Cyclers

Thermal cyclers used with the Exome Protocol must support uniform heating of 125 µl emulsion volumes. Thermal cyclers recommended for use with the Exome 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)

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
Exome Protocol - 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	Post GEM Recovery - 10 min Cleanup - Silane Beads - 35 min		STOP 4°C ≤72 h or –20°C ≤2 weeks
	7 h	Cleanup - SPRIselect - 20 min	QC - 50 min	STOP 4°C ≤72 h or –20°C ≤2 weeks
	8 h			
Day 2	1 h	Shearing** - 35 min End Repair & A-tailing Prep - 10 min		
	2 h	Adaptor Ligation Prep - 10 min	End Repair & A-tailing Incubation - 1 h Adaptor Ligation Incubation - 15 min	
	3 h	Cleanup - SPRIselect - 20 min Sample Index PCR Prep - 10 min	Sample Index PCR - 25 min	STOP 4°C ≤72 h
	4 h	Cleanup - SPRIselect - 20 min	QC - 50 min	STOP 4°C ≤72 h or –20°C long-term
	5 h	Dry-down Prep - 5 min	Library Dry-down - 1.5 h	
	6 h			
	7 h	Enrichment (Hybridization) Prep - 25 min	Hybridization - 16 - 24 h (overnight)	
Day 3	1 h	Capture Reagent Prep - 10 min Enrichment (Capture) - 1.5 h		
	2 h	Post Capture PCR Prep - 10 min	Post Capture PCR - 20 min	
	3 h	Cleanup - SPRIselect - 20 min QC & qPCR Quantification*		STOP 4°C ≤72 h or –20°C long-term

**Final QC (50 min) and qPCR Quantification (~ 1 h total) time not included.*

Demonstrated Protocol Step 1

HMW gDNA Extraction

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

1

1. HMW gDNA Extraction

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

This Demonstrated 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.

Demonstrated Protocol Step 2

GEM Generation & Barcoding

Partition template HMW gDNA across millions of GEMs for barcoding

2

2. GEM Generation & Barcoding

Getting Started! – 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* *Avoid more than 10 freeze-thaw cycles*	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 in the *Chromium™ Genome Reagent Kits v2 User Guide* (CG00043) 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 *Vortex, 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 (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* *Avoid more than 10 freeze-thaw cycles*	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 in the *Chromium™ Genome Reagent Kits v2 User Guide* (CG00043) 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 *Vortex, 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 1ng/µl in Buffer EB in an 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.

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.e). For example, multiply by 4.4 to set up 4 reactions with 10% excess [4+(4x0.1)=4.4].

For v2 (16 rxns) Kit			
Sample Master Mix	**Part Number**	**1 rxn (µl)**	**8 rxns + 10% excess (µl)**
Genome Reagent Mix	220123	89.5	788
Additive A	220074	3	26
Genome Enzyme Mix	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	220114	89.5	2363
Additive A	220093	3	79
Genome Enzyme Mix	220113	5	132
Total	-	**97.5**	**2574**

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

c) Dispense **10 µl** Denaturing Agent into a tube strip.

d) If the 1 ng/µl HMW gDNA is not already in a tube strip at room temperature from step 2.1, transfer at least 15 µl diluted HMW gDNA solution into a new tube strip using **wide-bore** pipette tips.

e) Slowly transfer **10 µl** diluted gDNA 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.

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

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

h) Slowly add **3 µl** denatured gDNA to **97.5 µl** pre-dispensed Sample Master Mix with a multi-channel pipette while on ice.

i) 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.

j) 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 the Chromium™ Genome User Guide 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

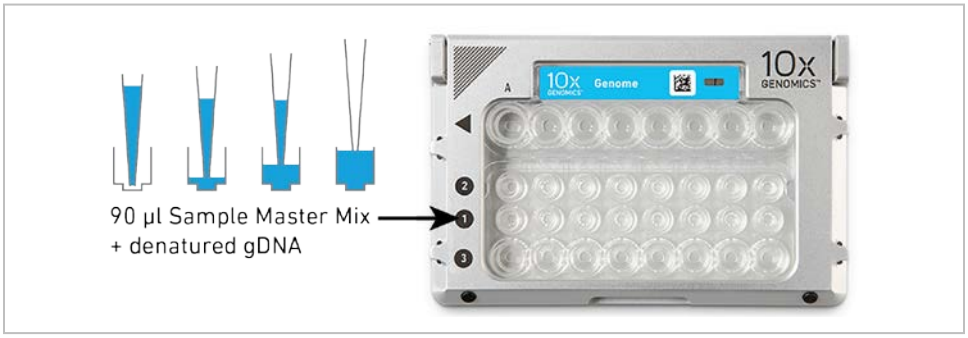
CRITICAL!

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 bubble, 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.

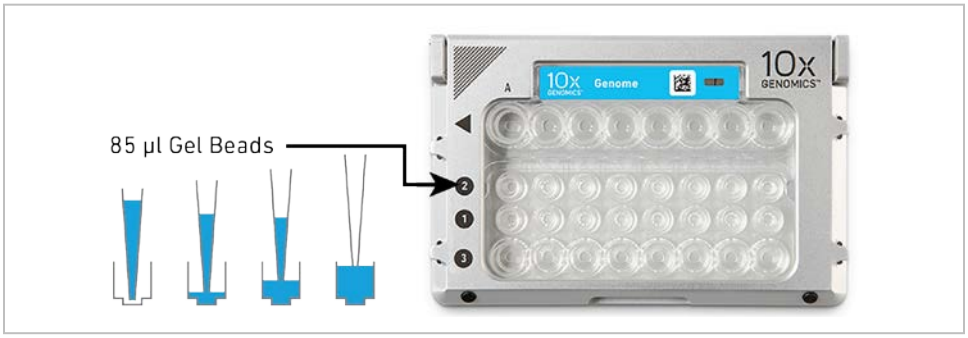
NOTE

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.

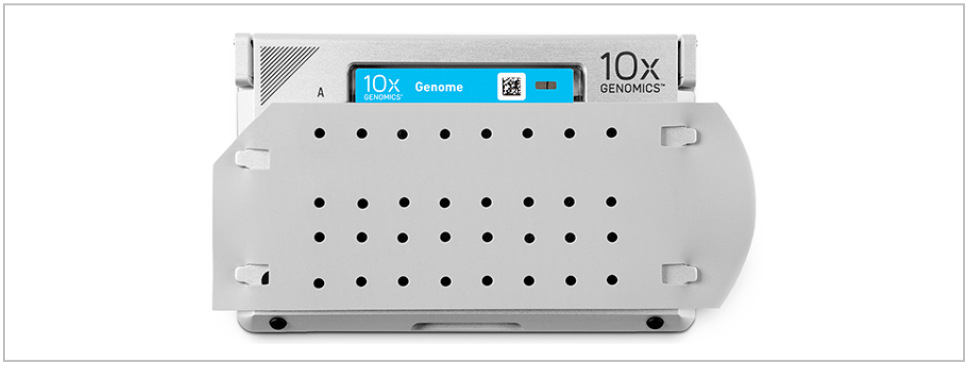


f) Pipette **270 µl** Partitioning Oil into the wells in the **row labeled 3**. Pipette Partitioning Oil into wells one at a time. Do not use a reagent reservoir for the Partitioning Oil. Do not add Partitioning Oil to any unused input wells that already contain 50% glycerol solution.

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 Eppendorf twin.tec® 96-Well PCR plate for GEM transfer on a chilled metal block resting on ice.

b) Press the eject button to eject the tray and remove the 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

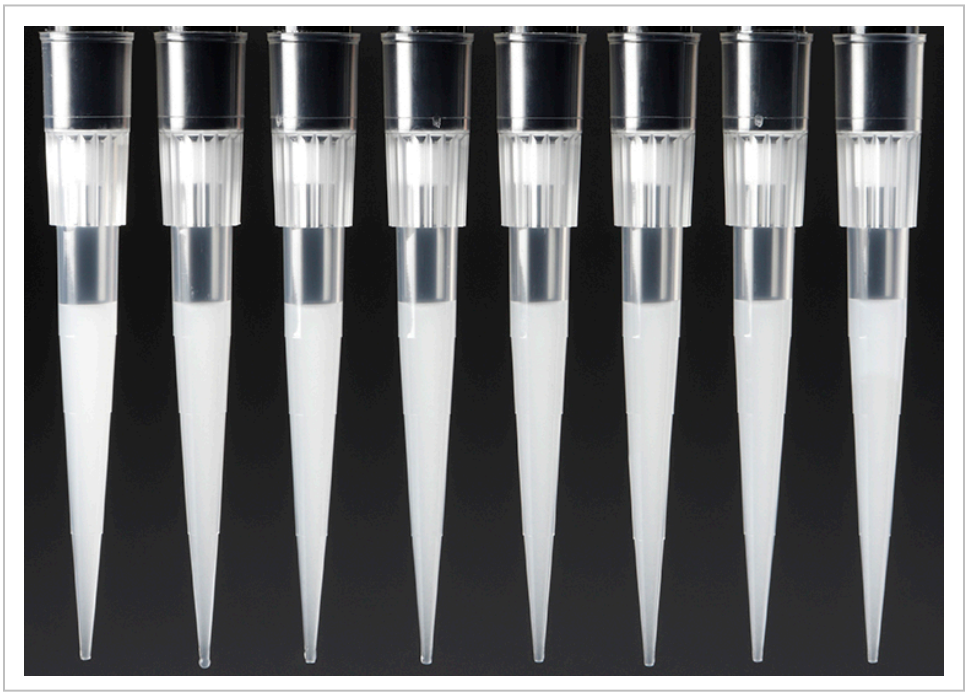
Abnormally high volume in any of the wells may indicate that a clog occurred during GEM generation.

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.



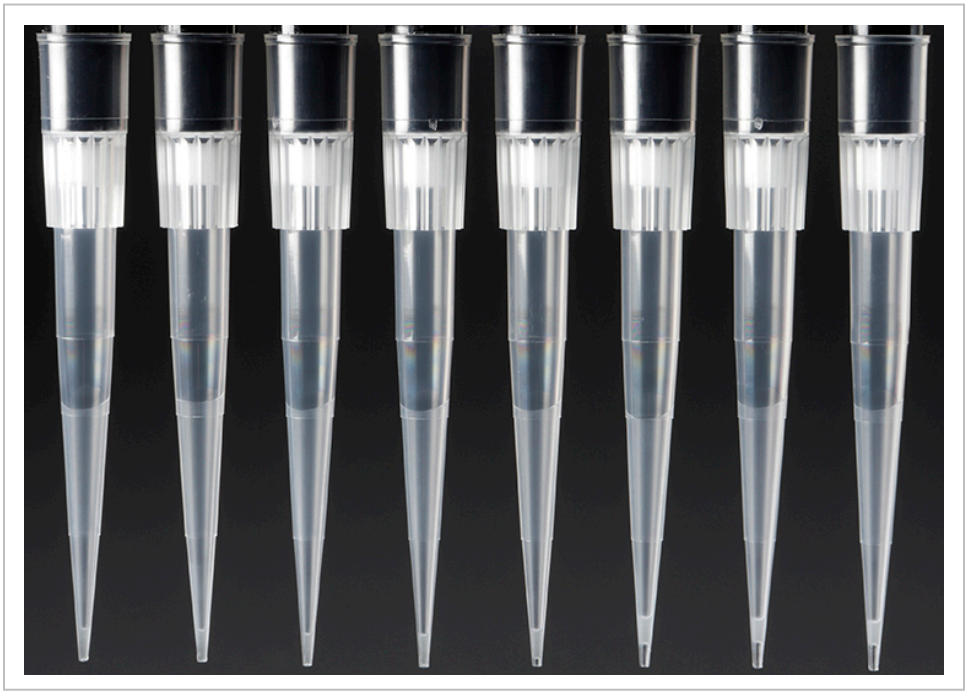
NOTE

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

g) Slowly dispense the GEMs into an Eppendorf twin-tec® 96-Well PCR 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 plate containing recovered GEMs on ice and seal the plate wells containing GEMs with Strip Caps 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 strip caps from the PCR plate with recovered GEMs. Check that the Plate Sealer plate block is at room temperature.

b) Seal the plate with pierceable foil heat seal at **185°C** for **6 sec** and promptly remove.

c) Load the sealed PCR plate into a thermal cycler 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 alternative 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 plate at **4°C** for up to **72 h** or at **–20°C** for up to **1 week**, or proceed directly to Post GEM Incubation Cleanup.

Demonstrated Protocol Step 3

Post GEM Incubation Cleanup & QC

Isolate and size DNA for library construction

3

3. Post GEM Incubation Cleanup & QC

Getting Started! – 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	-	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 80% Ethanol (20 ml for 8 samples)

Getting Started! – HT (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	-	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 80% Ethanol (60 ml for 24 samples)

Post GEM Incubation Cleanup & QC

3.1. Post GEM Incubation Cleanup – Silane DynaBeads

a) At room temperature, remove the foil seal and add **125 µl** Recovery Agent to each well containing post incubation GEMs. Pipette mix thoroughly and transfer the entire volume to an 8-tube strip.

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 5ul, 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.

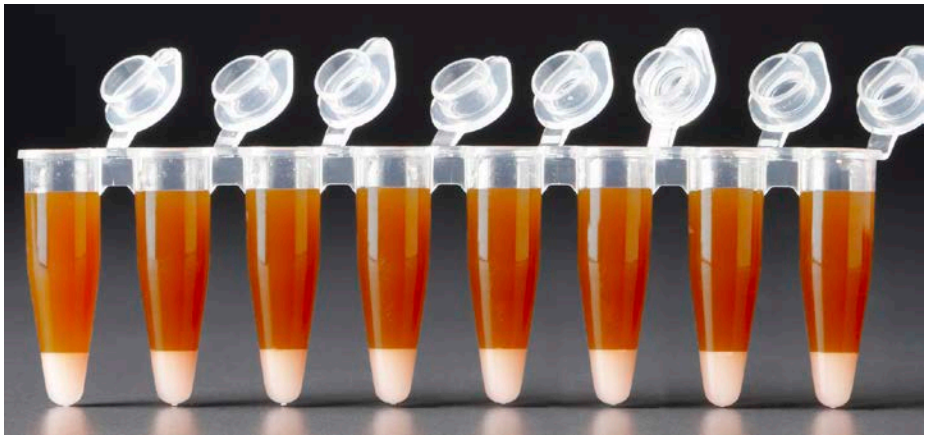
For v2 (16 rxns) Kit			
DynaBeads Cleanup Mix	**Part Number**	**1 rxn (µl)**	**8 rxns + 10% excess (µl)**
Buffer Sample Clean Up 1	220020	130	1144
DynaBeads MyOne Silane	-	14	123
Additive A	220074	6	53
Total	-	**150**	**1320**

For HT (96 rxns) Kit			
DynaBeads Cleanup Mix	**Part Number**	**1 rxn (µl)**	**24 rxns + 10% excess (µl)**
Buffer Sample Clean Up 1	220094	130	3432
DynaBeads MyOne Silane	-	14	370
Additive A	220093	6	158
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	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	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

CRITICAL!

This step is a series of two consecutive SPRIselect Cleanups.

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	-	196	1960
Additive A	220074	4	40
Total	-	**200**	**2000**

For HT (96 rxns) Kit			
Elution Solution II	**Part Number**	**1 rxn (µl)**	**24 rxns + 25% excess (µl)**
Buffer EB	-	196	5880
Additive A	220093	4	120
Total	-	**200**	**6000**

b) Vortex the SPRIselect Reagent until fully resuspended. Add **60 µl** SPRIselect Reagent (**1.2X**) 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 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.

Repeat

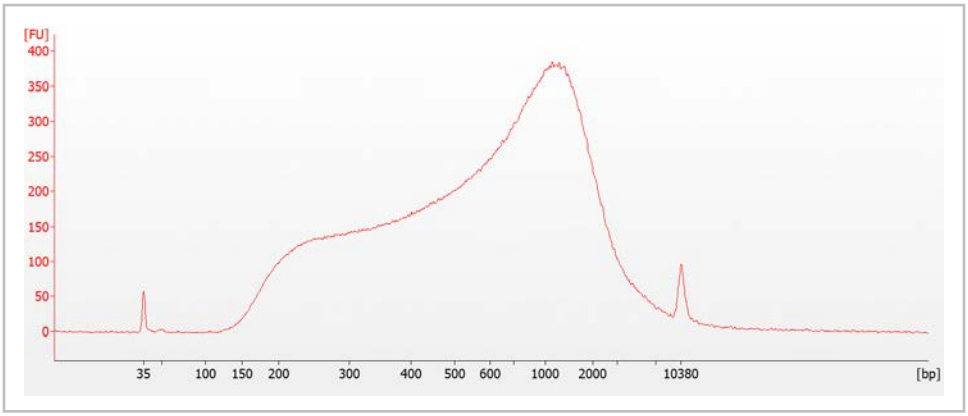
n) **Repeat** steps b-m for a total of two SPRIselect Cleanups.

STOP

o) 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 chip to determine yield and fragment size. Traces should resemble the overall shape of the sample electropherogram shown below.



Demonstrated Protocol Step 4

Library Construction

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

4. Library Construction

Getting Started! – 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	220124	–20°C
Adaptor Mix	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	220121	–20°C
DNA Ligase	220110	–20°C
Amplification Master Mix	220125	–20°C
Kapa DNA Quantification Kit for Illumina® Platforms	-	Manufacturer's recommendations

Prepare 80% Ethanol (10 ml for 8 samples)

Getting Started! – HT (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	220101	–20°C
Adaptor Mix	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	220116	–20°C
DNA Ligase	220118	–20°C
Amplification Master Mix	220119	–20°C
Kapa DNA Quantification Kit for Illumina® Platforms	-	Manufacturer's recommendations

Prepare 80% Ethanol (30 ml for 24 samples)

Library Construction

4.1. Shearing

a) Shear 50 µl of sample obtained from Post GEM Incubation Cleanup according to Covaris's recommended settings to achieve target peak size of 225 bp for a standard DNA sample.

b) Centrifuge tubes briefly and transfer 50 µl of sheared sample to a tube strip.

4.2. 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	220120	7.5	66
End Repair and A-tailing Enzyme	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	220115	7.5	198
End Repair and A-tailing Enzyme	220116	15	396
Total	-	**25**	**660**

c) Add **25 µl** End Repair and A-tailing Mix to each tube containing **50 µl** sample from Shearing. 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.

4.3. 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	220109	22	194
DNA Ligase	221110	11	97
Adaptor Mix	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	220117	22	580
DNA Ligase	220118	11	290
Adaptor Mix	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.

4.4. Post Ligation Cleanup – SPRIselect

a) Vortex the SPRIselect Reagent until fully resuspended. Add **198 µl** SPRIselect Reagent (**1.8X**) to each sample in the tube strip. Pipette mix thoroughly.

CRITICAL!

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

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 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.

4.5. Sample Index PCR

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	220125	50	440
Forward PCR Primer	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	220119	50	1320
Forward PCR Primer	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 a single 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 12 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, 11X (for 12 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.

4.6. Post Sample Index Cleanup – SPRI Select

a) Vortex the SPRIselect Reagent until fully resuspended. Add **180 µl** (**1.8X**) SPRIselect Reagent to each sample in the tube strip. Pipette mix thoroughly.

CRITICAL!

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

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 **20 µl** **Nuclease-Free Water**.

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

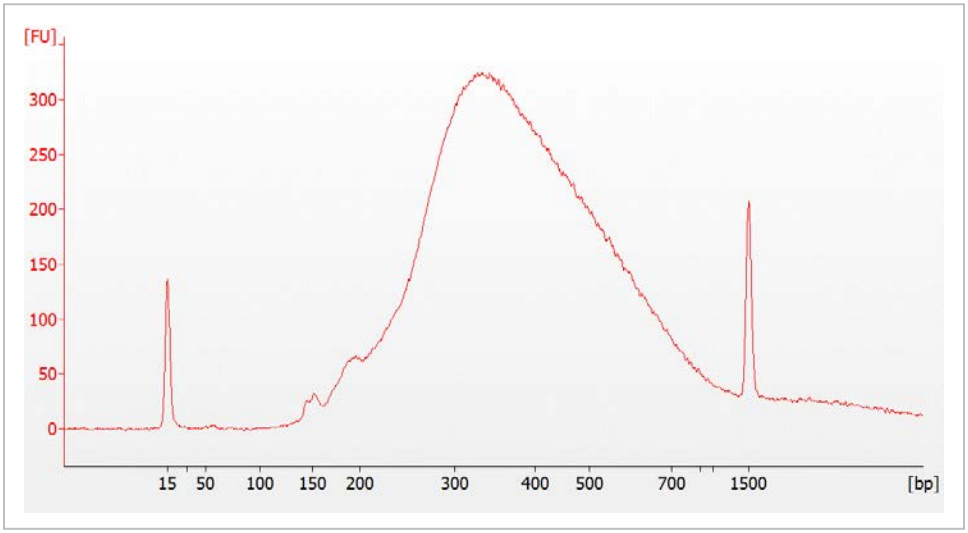
k) Centrifuge the tube strip briefly and return it to a 10x Magnetic Separator in the **Low** position until the solution is clear.

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

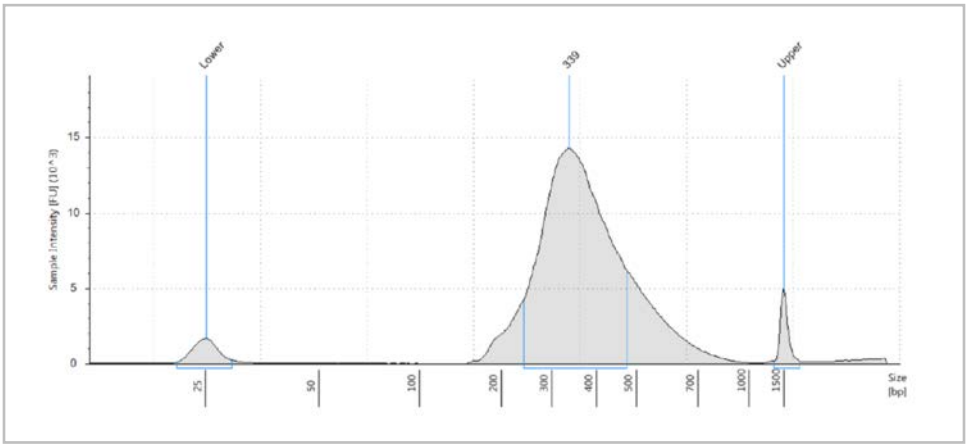
m) Store the tube strip at **4°C** for up to **72 h** or at **–20°C** for **long-term** storage.

4.7. Post Library Construction QC & Quantification

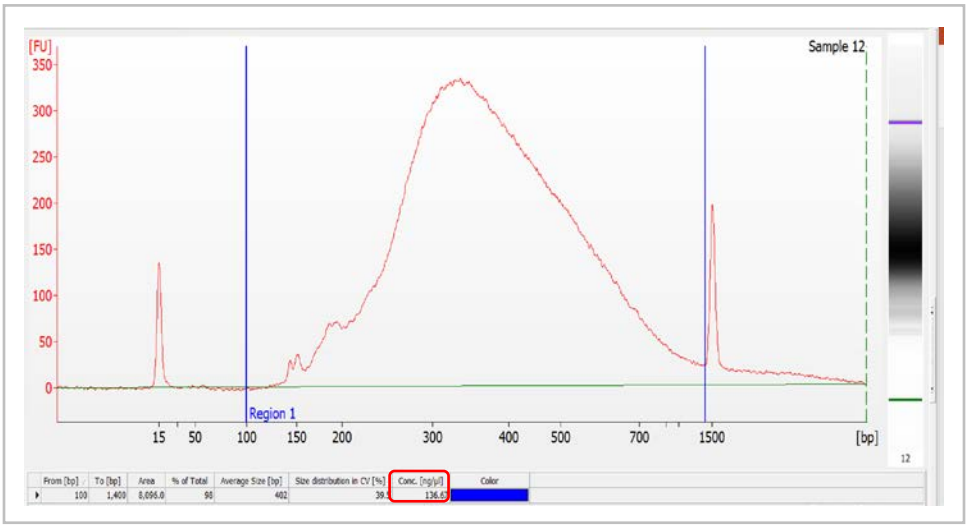
a) **EITHER** Run **1 µl** sample on the Agilent Bioanalyzer DNA 1000 chip to determine fragment size. Verify that a library has been generated by looking for a distribution similar to those illustrated below.



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 that illustrated below.



c) Measure the concentration of the library by performing a smear analysis for the region of 100 – 1400 bp. This concentration is then used to calculate the input volume of the library into target enrichment (Protocol Step 5).



Example Calculation for Target Enrichment (Hybridization) Input Volume:

Agilent 2100 Expert Concentration: 136.67 [ng/µl]

Volume for 1 µg input:

$$= \frac{\text{Mass (µg)} \times 1000 \left(\frac{\text{ng}}{\text{µg}}\right)}{\text{Concentration} \left(\frac{\text{ng}}{\text{µl}}\right)}$$

$$= \frac{1 \text{ (µg)} \times 1000 \left(\frac{\text{ng}}{\text{µg}}\right)}{137 \left(\frac{\text{ng}}{\text{µl}}\right)}$$

= 7.3 µl

Demonstrated Protocol Step 5

Target Enrichment

Hybridization: Isolate exome-specific fragments for sequencing

5

5. Target Enrichment (Hybridization)

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

Equilibrate to room temperature before use:

Item	Part Number	Storage Location
SureSelect Hyb 3 (yellow cap)	-	Manufacturer's recommendations

Place on ice:

Item	Part Number	Storage Location
SureSelect Indexing Block 1 (green cap)	-	Manufacturer's recommendations
SureSelect Block 2 (blue cap)	-	Manufacturer's recommendations
SureSelect RNAse block (purple cap)	-	Manufacturer's recommendations
SureSelectXT Human All Exon V6 Capture Library	-	Manufacturer's recommendations

Obtain:

Item	Part Number	Storage Location
SureSelect Hyb 1 (orange cap)	-	Manufacturer's recommendations
SureSelect Hyb 2 (red cap)	-	Manufacturer's recommendations
SureSelect Hyb 4 (black cap)	-	Manufacturer's recommendations
IDT xGen® Universal Blocking Oligo - TS-p5 *Universal Blocking Oligos should be resuspended per manufacturer's protocol to 1 µl / reaction*	-	Manufacturer's recommendations
IDT xGen® Universal Blocking Oligo - TS-p7(8nt) *Universal Blocking Oligos should be resuspended per manufacturer's protocol to 1 µl / reaction*	-	Manufacturer's recommendations

Target Enrichment (Hybridization)

5.1. Library Hybridization

Each DNA library is already indexed with appropriate indices, so addition of the sample index by PCR after hybridization is not necessary. Samples can also be multiplexed for hybridization.

NOTE

Use a multi-channel pipette where possible to minimize evaporation during sample handling, which can adversely affect enrichment efficiency. All incubation steps occurring at elevated temperatures are crucial to the success of target enrichment and should only be conducted in thermal cyclers with a heated lid. Avoid using thermomixers except for room temperature incubations.

a) Place **1 µg** of each DNA library as determined in Step 4.6, along with **1 µl** TS-p5 blocking oligo and **1 µl** TS-p7 (8nt) blocking oligo per library, into a separate well of a 96-well plate or 8-tube strip.

b) Use a vacuum concentrator to dehydrate the samples at **60°C** for **30 min**.

c) Visually inspect samples to verify that samples and blocking oligos are completely dried. If needed, dehydrate for additional 15 min intervals, until all samples are completely dried.

d) Reconstitute each lyophilized sample with **3.4 µl** Nuclease-Free Water.

e) Seal the wells, then mix by vortexing at **1500 rpm** for **5 min**. Centrifuge briefly.

f) Prepare the Hybridization Buffer by mixing the reagents shown below at room temperature. Vortex and centrifuge briefly.

For v2 (16 rxns) Kit			
Hybridization Mix	**Part Number**	**1 rxn (µl)**	**8 rxns + 1 rxn excess (µl)**
SureSelect Hyb 1 (orange cap)	-	6.63	59.7
SureSelect Hyb 2 (red cap)	-	0.27	2.4
SureSelect Hyb 3 (yellow cap)	-	2.65	23.9
SureSelect Hyb 4 (black cap)	-	3.45	31.0
Total	-	**13**	**117**

For HT (96 rxns) Kit			
Hybridization Mix	**Part Number**	**1 rxn (µl)**	**24 rxns + 2 rxns excess (µl)**
SureSelect Hyb 1 (orange cap)	-	6.63	172.4
SureSelect Hyb 2 (red cap)	-	0.27	7.0
SureSelect Hyb 3 (yellow cap)	-	2.65	68.9
SureSelect Hyb 4 (black cap)	-	3.45	89.7
Total	-	**13**	**338**

g) If precipitate forms, warm the Hybridization Buffer at **65°C** for **5 min**. Keep the prepared Hybridization Buffer at room temperature until ready to use.

h) Prepare the SureSelect Block Mix at room temperature. Vortex and centrifuge briefly.

For v2 (16 rxns) Kit			
Block Mix	**Part Number**	**1 rxn (µl)**	**8 rxns + 1 rxn excess (µl)**
Water	-	0.6	5.4
SureSelect Indexing Block 1 (green cap)	-	2.5	22.5
SureSelect Block 2 (blue cap)	-	2.5	22.5
Total	-	**5.6**	**50.4**

For HT (96 rxns) Kit			
Block Mix	**Part Number**	**1 rxn (µl)**	**24 rxns + 2 rxns excess (µl)**
Water	-	0.6	15.6
SureSelect Indexing Block 1 (green cap)	-	2.5	65
SureSelect Block 2 (blue cap)	-	2.5	65
Total	-	**5.6**	**145.6**

i) Add **5.6 µl** Block Mix to each reconstituted sample. Pipette mix thoroughly and centrifuge briefly

j) Place the 96-well plate or tube strip containing DNA samples with Block Mix in thermal cycler and start the SureSelect hybridization protocol.

Lid Temperature	**Reaction Volume**	
105°C	30 µl	
Step	**Temperature**	**Time**
1	95°C	5:00
2	65°C	5:00
3	65°C	Hold

k) Prepare the Capture Library (Bait) Mix by adding the reagents shown below. Vortex and centrifuge briefly. Place on ice until the thermal cycling program has reached the 65°C Hold step (step 3).

For v2 (16 rxns) Kit			
Capture Library (Bait) Mix	**Part Number**	**1 rxn (µl)**	**8 rxns + 1 rxn excess (µl)**
Water	-	1.5	13.5
RNase Block (purple cap)	-	0.5	4.5
SureSelect Baits	-	5	45
Total	-	**7**	**63**

For HT (96 rxns) Kit			
Capture Library (Bait) Mix	**Part Number**	**1 rxn (µl)**	**24 rxns + 2 rxns excess (µl)**
Water	-	1.5	39
RNase Block (purple cap)	-	0.5	13
SureSelect Baits	-	5	130
Total	-	**7**	**182**

l) Once the thermal cycling program is at the 65°C Hold step, add the Hybridization Buffer to the tube containing the Capture Library (Bait) Mix. Vortex and centrifuge briefly.

For v2 (16 rxns) Kit			
Capture Library Hybridization Mix	**Part Number**	**1 rxn (µl)**	**8 rxns + 1 rxn excess (µl)**
Capture Library (Bait) Mix	-	7	63
Hybridization Solution	-	13	117
Total	-	**20**	**180**

For HT (96 rxns) Kit			
Capture Library Hybridization Mix	**Part Number**	**1 rxn (µl)**	**24 rxns + 2 rxns excess (µl)**
Capture Library (Bait) Mix	-	7	182
Hybridization Solution	-	13	338
Total	-	**20**	**520**

m) Transfer **20 µl** aliquots of the Capture Library Hybridization Mix into a new plate or tube strip and centrifuge briefly.

n) Using a multi-channel P200 pipette, quickly transfer **20 µl** Capture Library Hybridization Mix into the DNA with blocker sample wells in the thermal cycler held at 65°C. Pipette mix 10 times and immediately cap the wells with new caps.

CRITICAL!

Sample wells must be uncapped for <20 sec.

o) Close thermal cycler lid and incubate the hybridization mixture for **16 h – 24 h** at **65°C**, with lid temperature at **105°C.**

Demonstrated Protocol Step 6

Target Enrichment

Capture: Isolate exome-specific fragments for sequencing

6. Target Enrichment (Capture)

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

Equilibrate to room temperature before use:

Item	Part Number	Storage Location
DynaBeads® MyOne™ Streptavidin T1 Beads	-	Manufacturer's recommendations
Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations
Post Capture PCR Primers	220037	–20°C
Agilent Bioanalyzer High Sensitivity Kit *If used for QC*	-	Manufacturer's recommendations
Agilent TapeStation D1000 ScreenTape and Reagents *If used for QC*	-	Manufacturer's recommendations

Place on ice:

Item	Part Number	Storage Location
Amplification Master Mix	220129	–20°C
Kapa DNA Quantification Kit for Illumina® Platforms	-	Manufacturer's recommendations

Obtain:

Item	Part Number	Storage Location
SureSelect Binding Buffer	-	Manufacturer's recommendations
SureSelect Wash Buffer 1	-	Manufacturer's recommendations
SureSelect Wash Buffer 2	-	Manufacturer's recommendations
Qiagen Buffer EB	-	Manufacturer's recommendations

Prepare 80% Ethanol (10 ml for 8 samples)

Target Enrichment (Capture)

6.1. Preparing Streptavidin Beads

Each DNA library is already indexed with appropriate indices, so addition of the sample index by PCR after hybridization is not necessary. Samples can also be multiplexed for hybridization.

NOTE

Use a multi-channel pipette where possible to minimize evaporation during sample handling, which can adversely affect enrichment efficiency.

All incubation steps occurring at elevated temperatures are crucial to the success of target enrichment and should only be conducted in thermal cyclers with a heated lid. Avoid using thermomixers except for room temperature incubations.

a) Aliquot **210 µl** SureSelect Wash Buffer 2 into a new plate or tube strips. Aliquot 3 wells of buffer for each DNA sample.

b) Place the sealed plate or tube strips in thermal cycler at **65°C** with the lid temperature at **80°C** until use in step 6.2.

c) Ensure Dynabeads MyOne Streptavidin T1 Beads are at **room temperature** before use. Vigorously vortex to resuspend the beads.

d) For each hybridization, wash **50 µl** Beads.

e) Wash Streptavidin T1 beads in bulk (Example below sufficient for **4 hybridizations,** adjust volumes according to number of samples being processed).

i. Dispense **210 µl** Dynabead MyOne Streptavidin T1 Beads.

ii. Add **840 µl** SureSelect Binding Buffer.

iii. Vortex mix to fully resuspend beads.

iv. Centrifuge briefly and place on a magnetic rack.

v. Wait until solution is clear, then remove and discard supernatant.

Repeat

vi. **Repeat** steps ii – v twice more for a total of 3 washes.

vii. Resuspend the beads in **840 µl** SureSelect Binding Buffer.

f) Aliquot 200 µl washed beads into each well of a new plate or tube strip.

6.2. Capturing Hybridized DNA & Washing

a) Maintain the hybridization plate or tube strip at 65°C and use a multi-channel pipette to transfer the entire volume (**~25 – 29 µl**) of each hybridization mixture to tube strip wells containing 200 µl washed streptavidin beads. Cap the wells.

b) Incubate the capture plate or tube strip on a 96-well plate mixer, mixing vigorously at **1500 rpm** for **30 min** at **room temperature**. Ensure the samples are properly mixing in the wells.

c) Centrifuge the plate or tube strip briefly.

d) Place the plate or tube strip in the **High** position of a 10x™ Magnetic Separator until the solution is clear.

e) Carefully remove and discard the supernatant.

f) Immediately resuspend the beads in **200 µl** SureSelect Wash Buffer 1 and pipette mix thoroughly. Cap the wells.

g) Incubate the capture plate or tube strip on a 96-well plate mixer, mixing vigorously at **1500 rpm** for **15 min** at **room temperature**.

h) Centrifuge the plate or tube strip briefly.

i) Place the plate or tube strip in the **High** position of a 10x Magnetic Separator until the solution is clear.

j) Carefully remove and discard the supernatant.

k) Immediately resuspend the beads in **200 µl** 65°C pre-warmed SureSelect Wash Buffer 2 and pipette mix thoroughly. Cap the wells.

l) Incubate the plate or tube strip for **10 min** at **65°C** on a thermal cycler.

CRITICAL! *It is important to maintain the resuspended beads at 65°C during the washing procedure to ensure capture specificity. Do not use a thermomixer.*

m) Centrifuge the plate or tube strip briefly.

n) Place the plate or tube strip in the High position of a 10x Magnetic Separator until the solution is clear.

o) Carefully remove and discard the supernatant.

Repeat p) **Repeat** steps k – o twice more for a total of 3 washes.

q) Centrifuge briefly and place the samples in the **Low** position of 10x Magnetic Separator, until the solution is clear. Remove and discard any remaining wash buffer.

r) Remove the plate or tube strip from the magnetic separator and immediately add **22.5 µl** Nuclease-Free Water to each sample well. Pipette mix until beads are fully resuspended.

s) Keep the samples on ice until ready to use in Post Capture PCR.

6.3. Post Capture PCR

a) Prepare the Post Capture PCR Mix by adding the reagents in the order shown below. Pipette mix thoroughly and centrifuge briefly.

NOTE

Ensure use of 10x™ Amplification Master Mix and Post Capture PCR Primers.

For v2 (16 rxns) Kit			
Post Capture PCR Mix	**Part Number**	**1 rxn (µl)**	**8 rxns + 10% excess (µl)**
Amplification Master Mix	220129	25	220
Post Capture PCR Primers	220037	2.5	22
Total	-	**27.5**	**242**

For HT (96 rxns) Kit			
Post Capture PCR Mix	**Part Number**	**1 rxn (µl)**	**24 rxns + 10% excess (µl)**
Amplification Master Mix	220129	25	660
Post Capture PCR Primers	220037	2.5	66
Total	-	**27.5**	**726**

b) Add **27.5 µl** Post Capture PCR Mix to each well containing 22.5 µl resuspended beads. Pipette mix thoroughly and centrifuge briefly.

NOTE

Always amplify the entirety of the captured library, rather than a subset of the captured library, to ensure maximum library diversity.

c) Run the Post Capture PCR protocol.

Lid Temperature	Reaction Volume	Run Time
105°C	50 µl	~20 min
Step	**Temperature**	**Time**
1	98°C	0:45
2	98°C	0:15
3	60°C	0:30
4	72°C	0:30
5	Go to Step 2, 7X (for 8 cycles in total)	
6	72°C	1:00
7	4°C	Hold

6.4. Post Capture PCR Cleanup – SPRIselect

a) Vortex the SPRIselect Reagent until fully resuspended. Add **90 µl** (**1.8X**) SPRIselect Reagent to each sample in the tube strip. Pipette mix thoroughly and centrifuge briefly.

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

f) Carefully remove and discard the ethanol wash.

Repeat

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

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

i) Remove and discard any remaining ethanol wash. Remove the tube strip from the magnet. Immediately add **20 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.

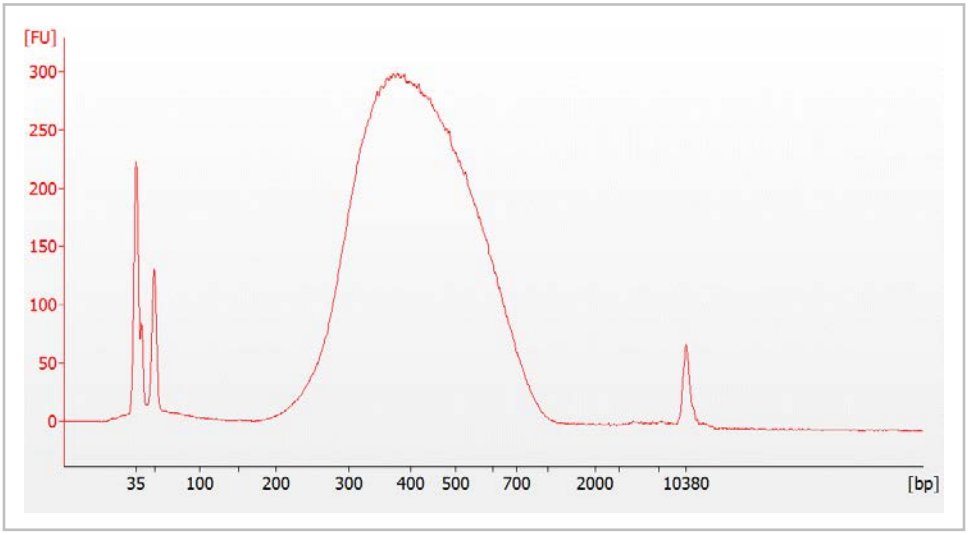
l) Transfer **20 µl** of sample to a new tube strip.

STOP

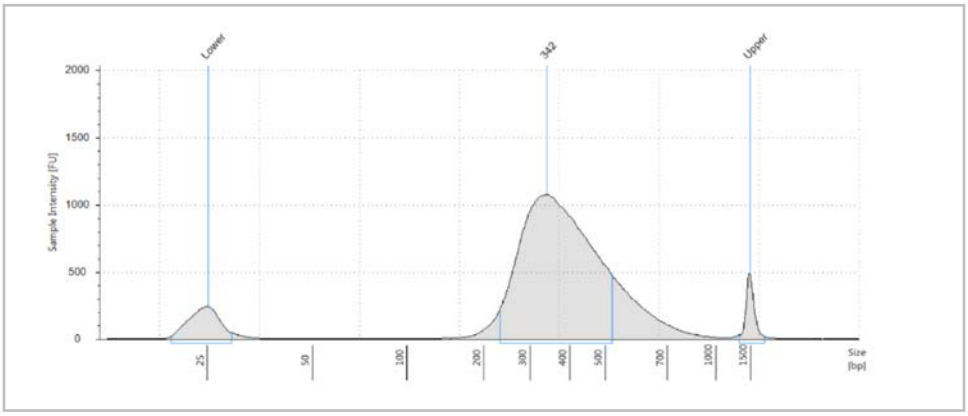
m) Store the tube strip at **4°C** for up to **72 h** or at **–20°C** for **long-term** storage.

6.5. Post Target Enrichment Library QC

a) **EITHER** Run **1 µl** sample on the Agilent Bioanalyzer High Sensitivity chip to determine fragment size. Peak of fragment positioned 320 – 420 bp (mean 400 – 475 bp).



b) **OR** Run **1 µl** sample on the Agilent TapeStation D1000 ScreenTape to determine fragment size. Peak of fragment positioned 320 – 420 bp (mean 400 – 475 bp).



6.6. Post Target Enrichment Library Quantification

NOTE

Typically a series of 1:40,000, 1:200,000, 1:1,000,000 and 1:5,000,000 of the completed Exome 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 96-well PCR Plate.

e) Add **4 µl** of sample dilutions and 4 µl DNA Standards to appropriate wells. Centrifuge the PCR plate briefly.

f) Run DNA Quantification Cycling Protocol with data acquisition at Step 3.

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

g) Follow the manufacturer's recommendations for qPCR-based quantification. For library quantification for sequencer clustering, determine the concentration using a fixed insert size of 420 bp.

Demonstrated Protocol Step 7

Sequencing

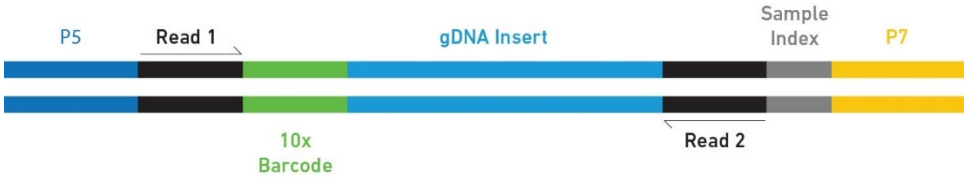
Sequencing prepared libraries

7

7. Sequencing Libraries

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

Exome 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.



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

7.1. Sequencing Depth Recommendations

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

Specifications	Recommended Coverage for Agilent SureSelect Human All Exon V6 Protocol
Gigabases of Sequence	9
Reads Passing Filter	90 Million (45 Million read pairs)
Targeted Deduped Depth	>60x

7.2. Sequencing Run Parameters

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

b) Exome 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 for Agilent SureSelect Human All Exon V6 Protocol
Read 1	100 cycles
i7 index	8 cycles
i5 index	0 cycles
Read 2	100 cycles

7.3. 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.