Chromium™

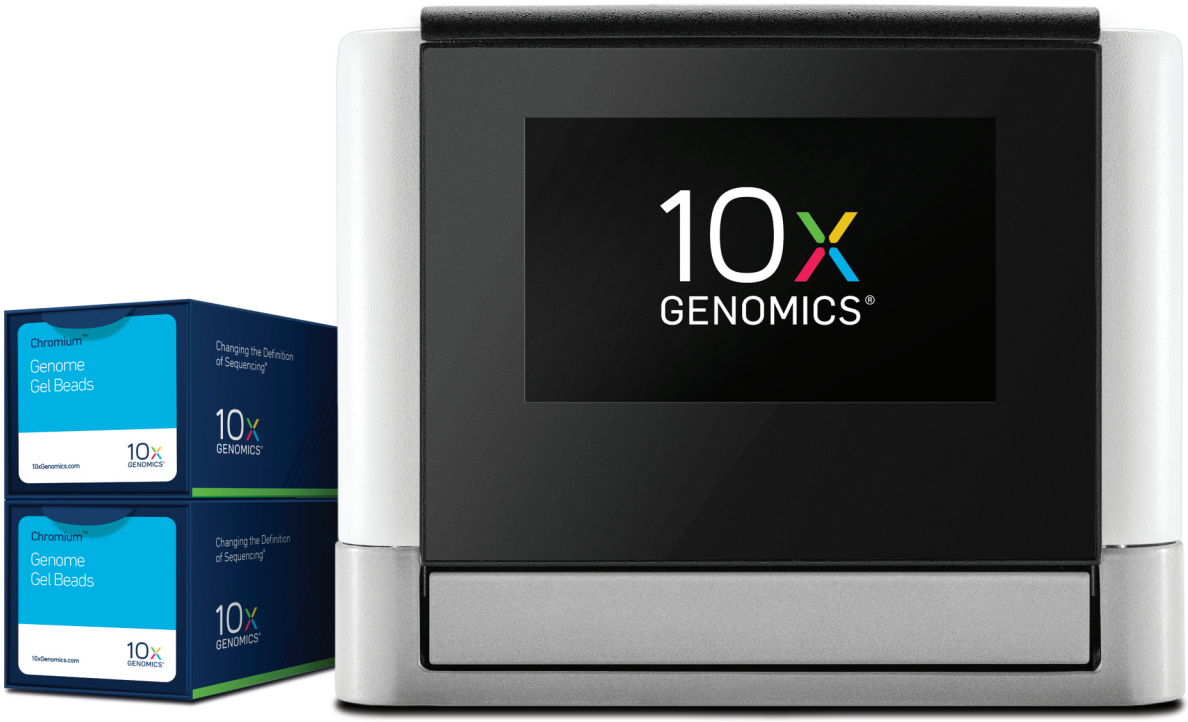
Genome Reagent Kits

Quick Reference Cards

FOR USE WITH

Chromium™ Genome Library, Gel Bead & Multiplex Kit, 16 rxns PN-120229

Chromium™ Genome Chip Kit PN-120216



1. gDNA Extraction

a) Dispense 1 x 10^6 live cells per extraction into a 2 ml microcentrifuge tube. Centrifuge 5 sec at 15,000 x g. Remove media leaving only the cell pellet behind

b) Add **200 µl room temperature PBS buffer**. Resuspend cell pellet by inverting 20x

c) Add **20 µl Proteinase K**. Mix by inverting tube 5x

d) Add **4 µl RNAse A** and **150 µl Buffer AL** to sample. Pulse-vortex mix 3x. Incubate 30 min at 25°C, centrifuge briefly

e) Vortex MagAttract® Suspension G for 1 min, add **15 µl** to sample

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

g) Centrifuge briefly, place on a DynaMag™-2 Magnetic Rack. Remove and discard supernatant

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

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

j) Centrifuge briefly and place on magnet. Remove and discard supernatant

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

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

m) Centrifuge briefly and place on magnet. Remove and discard supernatant

n) Add **700 µl nuclease-free water down the side of the tube opposite bead pellet**. Incubate **exactly 1 min**. Promptly remove and discard the supernatant

o) Repeat step n for a total of 2 washes

p) Remove sample from magnet. Add **150 µl Buffer AE** directly to bead pellet. Incubate at 25°C and 1400 rpm for 3 min

q) Centrifuge briefly and place on magnet

r) Transfer the supernatant to a new 1.5 ml low-bind screw-cap tube

s) Store at 4°C ≤2 weeks or at –20°C ≤6 months – or proceed directly to next step 

Chromium™ Genome

2. GEM Generation & Barcoding

10x GENOMICS®

Getting Started!

Equilibrate to room temperature before use:

- Qubit HS standards
- Additive A
- Denaturing Agent
- Surrogate Fluid (if <8 samples)
- Heat block from plate sealer
- Genome Gel Beads
- *Ensure no precipitate in liquid reagents before use*

Immediately before loading into chip:

- Vortex Gel Bead strip in a 10x™ Vortex Adapter and then flick sharply downwards to maximize Gel Bead recovery

Place on ice:

- Genome Reagent Mix
- Genome Enzyme Mix
- Chilled metal block

Obtain:

- Partitioning Oil
- Qiagen Buffer EB
- Genome Chip(s)
- 10x Chip Holder
- 10x Gasket(s)

2.1 Input gDNA Quantification

- Quantitate 3 µl extracted HMW gDNA solution (min 2 replicates) using the Qubit HS kit
- If extracted HMW gDNA stock >20 ng/µl, dilute to 5 ng/µl in Buffer EB. Mix 10x with a wide-bore tip. If stock <20 ng/µl, proceed directly to target concentration dilution
- Quantitate 3 µl of 5 ng/µl HMW gDNA solution (min 2 replicates)
- Dilute HMW gDNA solution to the target concentration indicated below in Buffer EB. Mix 10x with a wide-bore tip

	Genome Protocol	Exome Protocol
Target concentration	1 ng/µl	1.2 ng/µl
Acceptable range	0.8 - 1.2 ng/µl	1.0 - 1.4 ng/µl

- Quantitate 3 µl HMW gDNA solution (min 2 replicates) to confirm within acceptable range

2.2 Preparing Sample Master Mix

- Dispense **97.5 µl** Sample Master Mix into each well of a tube strip on ice
- Combine diluted HMW gDNA solution with **equal volume** Denaturing Agent. Mix 10x with wide-bore tip. Incubate mixture **5 min** at room temperature
- Add **2.5 µl** denatured HMW gDNA to each 97.5 µl Sample Master Mix in the tube strip
- Mix 10x with wide-bore tip
- Centrifuge briefly, maintain on ice

Sample Master Mix	1X (µl)	8.8X (µl)
Genome Reagent Mix	89.5	787.6
Additive A	3	26.4
Genome Enzyme Mix	5	44
Total	**97.5**	**858**

Chromium™ Genome

2. GEM Generation & Barcoding

2.3 Loading the Chromium™ Genome Chip

- If <8 samples, dispense **Surrogate Fluid** to each unused well: 90 µl - row labeled 1, 85 µl - row labeled 2, 270 µl - row labeled 3. Do not add Surrogate Fluid to Recovery Wells (row labeled ◀)
- Slowly dispense **90 µl** Sample Master Mix-denatured gDNA mix into bottom of row labeled **1**
- After vortexing and flicking the Gel Bead strip, slowly dispense **85 µl** Genome Gel Beads into bottom of row labeled **2**
- Dispense **270 µl** Partitioning Oil into row labeled **3**
- Attach 10x™ Gasket - notched cut top left, holes aligned with the wells

2.4 Running the Chromium Controller

- Press the touchscreen button to eject the tray
- Place assembled Chip, 10x Chip Holder and 10x Gasket on the tray
- Press the touchscreen button again to retract the tray
- Confirm Genome program on screen, press play button to start the run
- At run completion (~22 min), Chromium Controller will chime

2.5 Transferring GEMs

- Maintain an Eppendorf twin.tec® 96-well PCR plate on ice
- Remove and discard the 10x Gasket
- Open the 10x Chip Holder and fold the lid all the way back until it clicks
- Slowly aspirate **125 µl** GEMs from the lowest points of the Recovery Wells
- Dispense GEMs into the PCR plate on ice
- *If running >1 Chip, keep PCR plate on ice, seal wells with Strip Caps*

2.6 GEM Isothermal Incubation

- Seal PCR plate with foil heat seal at 185°C for 6 sec, promptly remove
- Place PCR plate in the thermal cycler and run the incubation program (right)
- Store in PCR plate at 4°C ≤72 h or at –20°C ≤2 weeks before next step

STOP

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

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3. Post GEM Incubation Cleanup & QC

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Getting Started!

Equilibrate to room temperature before use:

- Additive A
- DynaBeads® MyOne™ Silane beads
- Beckman Coulter SPRIselect Reagent
- Agilent Bioanalyzer High Sensitivity Kit

Thaw at 65°C:

- Buffer for Sample Cleanup 1 at 65°C for 10 min at max speed on a thermomixer. Cool to room temperature

Obtain:

- Recovery Agent
- Qiagen Buffer EB
- Bio-Rad 10% Tween 20

Prepare:

- 80% Ethanol (10 ml for 8 Genome Protocol samples)
- 80% Ethanol (20 ml for 8 Exome Protocol samples)

- *Ensure no precipitate in liquid reagents before use*

For Silane DynaBeads & SPRIselect Reagent Cleanups:

- *Vortex to fully resuspend Dynabeads and SPRIselect Reagent before adding to each sample in the tube strip*
- *Incubations are at room temperature. Mix = Pipette mix thouroughly*
- *Magnet H or L = Place in a 10x™ Magnetic Separator in the High or Low position*
- *Supernatant and ethanol wash should be discarded except where noted*

3.1 Post GEM Incubation Cleanup - Silane DynaBeads

- Remove foil seal
- Add **125 µl** Recovery Agent
- Mix. Transfer entire volume to tube strip, cap wells
- Vortex 15 sec in a 10x™ Vortex Clip. Centrifuge briefly
- Remove **135 µl** Recovery Agent/Partitioning Oil, discard
- Add **150 µl** DynaBeads Cleanup Mix
- Mix. Incubate **10 min**. Make Elution Solution I
- Magnet **H**. Remove supernatant
- Add **250 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- Add **200 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- Centrifuge briefly. Magnet **L**
- Remove remaining ethanol
- Remove from magnet
- Add **50 µl Elution Solution I**
- Incubate **30 sec**. Resuspend pellet in solution
- Incubate **5 min**
- Centrifuge briefly. Magnet **L**
- Transfer sample to new tube strip

Genome Protocol		
DynaBeads Cleanup Mix	**1X (µl)**	**8.8X (µl)**
Buffer for Sample Cleanup 1	140	1232
DynaBeads MyOne Silane	4	35
Additive A	6	53
Total	**150**	**1320**

Exome Protocol		
DynaBeads Cleanup Mix	**1X (µl)**	**8.8X (µl)**
Buffer for Sample Cleanup 1	130	1144
DynaBeads MyOne Silane	14	123
Additive A	6	53
Total	**150**	**1320**

Elution Solution I	**1 rxn (µl)**	**10 rxns (µl)**
Buffer EB	89	890
10% Tween 20	1	10
Additive A	10	100
Total	**100**	**1000**

3.2 Post GEM Incubation Cleanup - SPRIselect

Genome Protocol

- Add **35 µl** SPRIselect Reagent. Mix. Centrifuge briefly
- Incubate **5 min**. Make Elution Solution II
- Magnet **H**. Remove supernatant
- Add **125 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- **Repeat** 2 steps above, total of 2 washes
- Centrifuge briefly. Magnet **L**
- Remove remaining ethanol
- Remove from magnet
- Add **52 µl Elution Solution II**. Mix. Incubate **5 min**
- Centrifuge briefly. Magnet **L**
- Transfer **52 µl** sample to new tube strip
- SPRIselect cleanup complete
- Store at 4°C ≤72 h or at –20°C ≤2 weeks

STOP

Exome Protocol

- Add **60 µl** SPRIselect Reagent. Mix. Centrifuge briefly
- Incubate **5 min**. Make Elution Solution II
- Magnet **H**. Remove supernatant
- Add **125 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- **Repeat** 2 steps above, total of 2 washes
- Centrifuge briefly. Magnet **L**
- Remove remaining ethanol
- Remove from magnet
- Add **52 µl Elution Solution II**. Mix. Incubate **5 min**
- Centrifuge briefly. Magnet **L**
- Transfer **52 µl** sample to new tube strip
- **Repeat** all steps for total of 2 cleanups
- Store at 4°C ≤72 h or at –20°C ≤2 weeks

STOP

Genome Protocol		
Elution Solution II	**1 rxn (µl)**	**10 rxns (µl)**
Buffer EB	98	980
● Additive A	2	20
Total	**100**	**1000**

Exome Protocol		
Elution Solution II	**1 rxn (µl)**	**10 rxns (µl)**
Buffer EB	196	1960
● Additive A	4	40
Total	**200**	**2000**

3.3 Post GEM QC

- Run 1 µl Genome or Exome Protocol sample on the Agilent Bioanalyzer High Sensitivity chip to determine yield and fragment size

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4. Library Construction

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Getting Started!

Equilibrate to room temperature before use:

- End Repair and A-tailing Buffer
- Forward PCR Primer
- Adaptor Mix
- Ligation Buffer
- Genome Sample Index Plate
- Beckman Coulter SPRIselect Reagent
- Agilent Bioanalyzer DNA 1000 Kit, or
- Agilent TapeStation D1000 ScreenTape & Reagents
- Kapa DNA Quantification Kit for Illumina Platforms

Place on ice:

- End Repair and A-tailing Enzyme
- DNA Ligase
- PCR Master Mix

Obtain:

- Qiagen Buffer EB

Prepare:

- 80% Ethanol (10 ml for 8 samples)

- *Ensure no precipitate in liquid reagents before use*

For SPRIselect Reagent Cleanups:

- *Vortex to fully resuspend SPRIselect Reagent before adding to each sample in the tube strip*
- *Incubations are at room temperature*
- *Mix = Pipette mix thoroughly*
- *Magnet H or L = Place in a 10x™ Magnetic Separator in the High or Low position*
- *Supernatant and ethanol wash should be discarded except where noted*

4.1 Shearing (Exome Protocol Only)

- Shear **50 µl** sample from Post GEM Incubation Cleanup according to Covaris's recommended settings for the instrument and tube - target peak size of 225 bp, standard DNA sample
- Centrifuge tubes briefly and transfer **50 µl** sheared sample to a new tube strip

4.2 Library Construction: End Repair & A-tailing

End Repair and A-tailing Mix	1X (µl)	8.8X (µl)
End Repair & A-tailing Buffer	7	62
End Repair & A-tailing Enzyme	3	26
Total	**10**	**88**

Lid Temperature	Reaction Volume	Run Time
85°C	60 µl	~ 1h 5 min

Step	Temperature	Time
End Repair	20°C	30:00
A-tailing	65°C	30:00
Hold	4°C	Hold

- Add **10 µl** End Repair and A-tailing Mix to each tube containing 50 µl sample from Post GEM Incubation Cleanup (Genome Protocol) OR shearing (Exome Protocol)
- Pipette mix thoroughly and centrifuge briefly
- Place tube strip in the thermal cycler and run the End Repair and A-tailing program (right)

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4. Library Construction

4.3 Library Construction: Adaptor Ligation

Adaptor Ligation Mix	1X (µl)	8.8X (µl)
Nuclease-Free Water	7.5	66
Ligation Buffer	30	264
DNA Ligase	10	88
Adaptor Mix	2.5	22
Total	**50**	**440**

Lid Temperature	Reaction Volume	Run Time
30°C	110 µl	-
Step	**Temperature**	**Time**
1	20°C	15:00

- Add **50 µl** Adaptor Ligation Mix to each tube containing 60 µl sample from the End Repair and A-tailing step
- Pipette mix thoroughly and centrifuge briefly
- Place tube strip in the thermal cycler and run the Ligation program (above)

4.4 Post Ligation Cleanup - SPRIselect

Genome Protocol

- Add **88 µl** SPRIselect Reagent
- Mix. Incubate RT **5 min**
- Magnet **H**. Remove supernatant
- Add **250 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- **Repeat** 2 steps above, total of 2 washes
- Centrifuge briefly. Magnet **L**
- Remove remaining ethanol
- Remove from magnet
- Add **40 µl Buffer EB.** Mix. Incubate RT **5 min**
- Centrifuge briefly. Magnet **L**
- Transfer **40 µl** sample to new tube strip

Exome Protocol

- Add **198 µl** SPRIselect Reagent
- Mix. Incubate RT **5 min**
- Magnet **H**. Remove supernatant
- Add **250 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- **Repeat** 2 steps above, total of 2 washes
- Centrifuge briefly. Magnet **L**
- Remove remaining ethanol
- Remove from magnet
- Add **40 µl Buffer EB.** Mix. Incubate RT **5 min**
- Centrifuge briefly. Magnet **L**
- Transfer **40 µl** sample to new tube strip

4.5 Sample Index PCR

Sample Index PCR Mix	1X (µl)	8.8X (µl)
PCR Master Mix	50	440
Forward PCR Primer	5	44
Total	**55**	**484**

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~20 - 25 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 - Genome Protocol	Go to Step 2, 7X (for 8 cycles total)	
5 - Exome Protocol	Go to Step 2, 11X (for 12 cycles total)	
6	72°C	1:00
7	4°C	Hold

- Add **55 µl** Sample Index PCR Mix into each tube containing 40 µl purified Post Ligation sample
- Add **5 µl** of a single Genome Sample Index to each well and record assignment
- Pipette mix thoroughly and centrifuge briefly
- Place tube strip in the thermal cycler and run the program (right)
- Store at 4°C ≤72 h or proceed to next step

STOP

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4. Library Construction

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4.6 Post Sample Index PCR Cleanup - SPRIselect

Genome Protocol

- Add **50 µl** SPRIselect Reagent
- Mix. Incubate **5 min**
- Magnet **H**
- **DO NOT discard supernatant**
- **Transfer supernatant to new tube strip**
- Discard old tube strip
- Add **20 µl** SPRIselect Reagent
- Mix. Incubate **5 min**
- Magnet **H**. Remove supernatant
- Add **200 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- **Repeat** 2 steps above, total of 2 washes
- Centrifuge briefly. Magnet **L**
- Remove remaining ethanol
- Remove from magnet
- Add **20 µl Buffer EB.** Mix. Incubate **5 min**
- Centrifuge briefly. Magnet **L**
- Transfer **20 µl** sample to new tube strip
- Store at 4°C ≤72 h or –20°C for long-term storage STOP

Exome Protocol

- Add **180 µl** SPRIselect Reagent
- Mix. Incubate **5 min**
- Magnet **H**. Remove supernatant
- Add **250 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- **Repeat** 2 steps above, total of 2 washes
- Centrifuge briefly. Magnet **L**
- Remove remaining ethanol
- Remove from magnet
- Add **20 µl Nuclease-Free Water.** Mix. Incubate **5 min**
- Centrifuge briefly. Magnet **L**
- Transfer **20 µl** sample to new tube strip
- Store at 4°C ≤72 h or –20°C for long-term storage STOP

4.7 Post Library Construction QC

- Either run 1 µl Genome or Exome Protocol sample on the Agilent Bioanalyzer DNA 1000 chip to determine yield and fragment size. Measure the concentration of the library by integrating the region under the peak

	Genome Protocol	Exome Protocol
Peak of fragment	620 - 680 bp	320 - 380 bp

- Or run 1 µl Genome or Exome Protocol sample on the Agilent TapeStation D1000 ScreenTape to determine yield and fragment size. Measure the concentration of the library by integrating the region under the peak

4.8 Post Library Construction Quantification (Genome Protocol)

- Quantify libraries using Kapa DNA Quantification Kit for Illumina platforms following manufacturer's recommendations

Chromium™ Genome

5. Target Enrichment (Hybridization)

Exome Protocol Only

Getting Started!

Equilibrate to room temperature before use:

- SureSelect Hyb 1 bottle
- SureSelect Hyb 2
- SureSelect Hyb 3
- SureSelect Hyb 4
- SureSelect Index Block #1
- SureSelect Block #2

Place on ice:

- SureSelect baits
- RNAse block

Obtain:

- SureSelect Capture Reagents
- IDT xGen® Universal Blocking Oligo - TS-p5
- IDT xGen® Universal Blocking Oligo - TS-p7(8nt)

Resuspension:

- Universal Blocking Oligos resuspended per manufacturer's protocol to 1 µl / reaction
- *Ensure no precipitate in liquid reagents before use*

5.1 Library Hybridization

- Dehydrate **1 µg library** with 1 µl TS-p5 blocking oligo and 1 µl TS-p7(8nt) blocking oligo at 60°C in a vacuum concentrator
- Reconstitute with **3.4 µl** Nuclease-Free Water
- Mix at 1500 rpm for 5 min, centrifuge briefly
- Prepare Hybridization Solution at room temperature
- Prepare Blocking Mix at room temperature
- Add **5.6 µl** Blocking Mix to each reconstituted sample. Pipette mix, centrifuge briefly
- Place tube strip in thermal cycler and run the SureSelect hybridization program (right)
- Let tubes sit in the thermal cycler ≤5 min at 65°C
- Prepare the Capture Baits at RT. Place on ice until thermal cycling protocol has reached Step 3
- Add Hybridization Solution to Capture Baits. Pipette mix, centrifuge briefly
- Aliquot **20 µl** Capture Baits/Hybridization Solution into a new tube strip, centrifuge briefly
- Quickly and simultaneously transfer **20 µl** Capture Baits/Hybridization Solution to tubes at 65°C. Pipette mix 10x
- Cap tube strip with new caps, insert Bio-Rad tube frame and MicroAmp compression pad. Close thermal cycler lid
- After 16 h (up to 24 h), proceed to next step

Hybridization Solution	1X (µl)	8.8X (µl)
SureSelect Hyb 1 bottle	6.63	63.6
SureSelect Hyb 2	0.27	2.6
SureSelect Hyb 3	2.65	25.4
SureSelect Hyb 4	3.45	33.1
Total	**13**	**125**

Blocking Mix	1X (µl)	8.8X (µl)
Water	0.6	5.3
SureSelect Index Block #1	2.5	22.0
SureSelect Block #2	2.5	22.0
Total	**5.6**	**49.3**

Lid Temperature	Reaction Volume	Run Time
105°C	30 µl	-

Step	Temperature	Time
1	95°C	5:00
2	65°C	5:00
3	65°C	Hold

Capture Baits	1X (µl)	8.8X (µl)
Water	1.5	13.2
RNase Block	0.5	4.4
SureSelect Baits	5	44
Total	**7**	**61.6**

	1X (µl)	8.8X (µl)
Hybridization Solution	13	114.4

Chromium™ Genome

6. Target Enrichment (Capture)

Exome Protocol Only

10x GENOMICS®

Getting Started!

Equilibrate to room temperature before use:

- Post Capture PCR Master Mix
- Post Capture PCR Primers
- Beckman Coulter SPRIselect Reagent
- Agilent Bioanalyzer High Sensitivity Kit, or
- Agilent TapeStation High Sensitivity D1000 ScreenTape and Reagents
- Kapa DNA Quantification Kit for Illumina Platforms

- *Ensure no precipitate in liquid reagents before use*

Obtain:

- SureSelect Capture Reagents
- DynaBeads® MyOne™ Streptavidin T1 beads
- Qiagen Buffer EB

Warm to 65°C:

- Thermal cycler or heat block for tube strips

Prepare:

- 80% Ethanol (10 ml for 8 samples)

6.1 Preparing Streptavidin Beads

- Aliquot **210 µl** SureSelect Wash Buffer 2 into each of 3 tube strips
- Place tube strips in thermal cycler at 65°C with lid at 80°C until use in Step 6.2
- Wash **50 µl** beads for each hybridization (wash in bulk, see example to right)
- Aliquot **200 µl** beads into labeled tube strip wells

Bead washing for 4 hybridizations, adjust volumes accordingly for number of samples:

- Add **210 µl** Dynabead MyOne Streptavidin T1 beads (fully resuspended) to 1.6 ml eppendorf tube
- Add **840 µl** SureSelect Binding Buffer, vortex
- Centrifuge briefly, place on magnet, remove supernatant
- Repeat 2 steps above 2x more, total of 3 washes
- Resuspend beads in **840 µl** SureSelect Binding Buffer

6.2 Capturing Hybridized DNA & Washing

- Maintain the tube strip at 65°C while using a multichannel pipette to transfer the entire volume (~25 – 29 µl) of each hybridization mixture to tube strip wells containing 200 µl of washed Streptavidin beads. Cap wells
- Incubate tube strip on a 96-well plate mixer, mixing at 1500 rpm, **room temperature, 30 min**
- Centrifuge briefly. Magnet **H**. Remove supernatant
- Resuspend beads in **200 µl SureSelect Wash Buffer 1**. Pipette mix thoroughly. Cap wells
- Incubate tube strip on a 96-well plate mixer, mixing at 1500 rpm, **room temperature, 15 min**
- Centrifuge briefly, Magnet **H**. Remove supernatant
- Resuspend beads in **200 µl pre-warmed SureSelect Wash Buffer 2**. Pipette mix thoroughly. Cap wells
- Incubate tube strip on a 96-well plate mixer, mixing at 1500 rpm, **65°C, 10 min**
- Centrifuge briefly. Magnet **H**. Remove supernatant
- **Repeat** pre-warmed SureSelect Wash Buffer 2 wash 2x more, total of 3 washes
- Remove and discard any remaining wash buffer. Remove from magnet
- Add **22.5 µl Nuclease-Free Water**. Pipette mix until beads are fully resuspended
- Proceed immediately to next step

Chromium™ Genome

6. Target Enrichment (Capture)

Exome Protocol Only

6.3 Post Capture PCR

Post Capture PCR Mix	1X (µl)	8.8X (µl)
Post Capture PCR Master Mix	25	220
Post Capture PCR Primers	2.5	22
Total	**27.5**	**242**

- Dispense **27.5 µl** Post Capture PCR Mix into each tube containing 22.5 µl resuspended beads
- Pipette mix thoroughly and centrifuge briefly
- Place tube strip in the thermal cycler and run the program (right)

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 total)	
6	72°C	2:00
7	4°C	Hold

6.4 Post Capture PCR Cleanup - SPRIselect

- Add **90 µl** SPRIselect Reagent. Mix. Centrifuge briefly
- Incubate **5 min**
- Magnet **H**. Remove supernatant
- Add **200 µl** 80% ethanol to pellet
- Stand **30 sec**. Remove ethanol wash
- **Repeat** 2 steps above, total of 2 washes
- Centrifuge briefly. Magnet **L**
- Remove remaining ethanol
- Remove from magnet
- Add **20 µl Buffer EB.** Mix. Incubate **5 min**
- Centrifuge briefly. Magnet **L**
- Transfer **20 µl** sample to new tube strip
- Store at 4°C ≤72 h or –20°C for long-term storage STOP

For SPRIselect Reagent Cleanups:

- *Vortex to fully resuspend SPRIselect Reagent before adding to each sample in the tube strip*
- *Incubations are at room temperature*
- *Mix = Pipette mix thoroughly*
- *Magnet H or L = Place in a 10x™ Magnetic Separator in the High or Low position*
- *Supernatant and ethanol wash should be discarded except where noted*

6.5 Post Target Enrichment QC

- Either run 1 µl Exome Protocol sample on the Agilent Bioanalyzer High Senstivity chip to determine yield and fragment size. Measure the concentration of the library by integrating the region under the peak. Peak of fragment positioned 320 – 420 bp (mean 400 – 475 bp)
- Or run 2 µl Exome Protocol sample on the Agilent High Sensitivity D1000 ScreenTape to determine yield and fragment size. Measure the concentration of the library by integrating the region under the peak

6.6 Post Target Enrichment Library Quantification

- Quantify libraries using Kapa DNA Quantification Kit for Illumina platforms following manufacturer's recommendations

7. Sequencing

– The Genome Solution produces Illumina-ready sequencing libraries. See the Chromium™ Genome Reagent Kit User Guide (Document CG00022, Section 7) for further information

Sequencing Depth Recommendations

– The sequencing performance of Genome and Exome libraries is driven by both sequencing coverage and total number of reads

Specifications	Recommended Coverage for Genome Protocol (Human)	Recommended Coverage for Exome Protocol (Human)
Gigabases of Sequence	128	9
Reads Passing Filter	850 Million (425 Million read pairs)	84 Million (42 Million read pairs)
Targeted Deduped Depth	>30x	>60x

Sequencing Run Parameters

– Genome and Exome libraries use standard Illumina sequencing primers for both sequencing and index reads, and require no custom primers

– Genome and Exome libraries are run using paired-end sequencing with single indexing

Sequencing Read	Recommended Number of Cycles for Genome Protocol	Recommended Number of Cycles for Exome Protocol
Read 1	150 cycles	114 cycles
i7 index	8 cycles	8 cycles
i5 index	0 cycles	0 cycles
Read 2	150 cycles	98 cycles

Changing the Definition of Sequencing™

Legal Notices

Chromium™ Genome

Genome & Exome Protocols