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Chromium™

Genome HT Reagent Kits v2

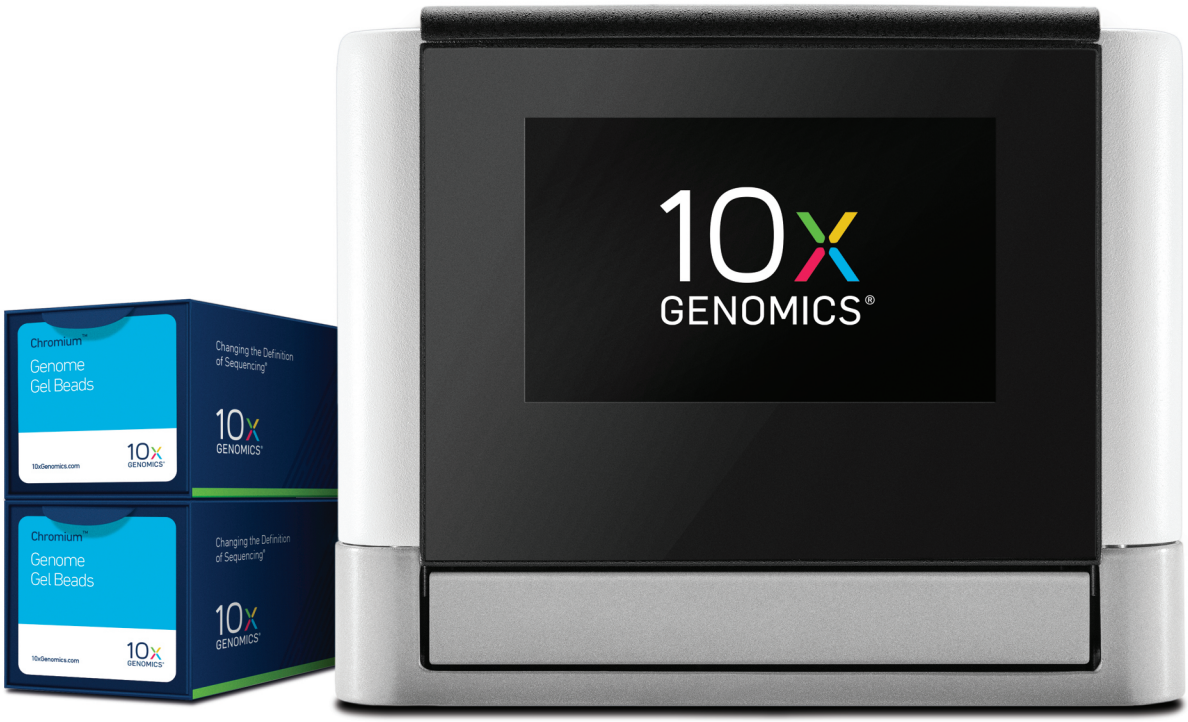
Quick Reference Cards

FOR USE WITH

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

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

Chromium™ i7 Multiplex Kit PN-120262



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

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.

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) Using a **wide-bore** tip, 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 

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2. GEM Generation & Barcoding

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Getting Started!

Equilibrate to room temperature before use:

- Qubit HS standards
- Additive A
- Denaturing Agent
- Genome Gel Beads
- *Ensure no precipitate in liquid reagents before use*

Place on ice:

- Genome Reagent Mix
- Genome Enzyme Mix
- Chilled Metal Block

Immediately before loading into chip:

- Centrifuge Genome Gel Bead Plate at **300 rcf** for **30 sec**
- Puncture the foil seal & pipette mix **20x** before transfer

Obtain:

- 50% Glycerol Solution (if < 8 samples)
- Partitioning Oil
- Genome Chip(s)
- 10x Chip Holder
- 10x Gasket(s)
- Qiagen Buffer EB
- Heat block from plate sealer

2.1 Input HMW 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 <20 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 <20 ng/µl HMW gDNA solution (min 2 replicates)
- Dilute HMW gDNA solution to the 1 ng/µl in Buffer EB. Mix 10x with a **wide-bore** tip
- Quantitate 3 µl HMW gDNA solution (min 2 replicates) to confirm within acceptable range of 0.8 - 1.2 ng/µl before proceeding to Preparing Sample Master Mix

2.2 Preparing Sample Master Mix

- Dispense **97.5 µl** Sample Master Mix into each well of a tube strip on ice
- Dispense **10 µl** Denaturing Agent into each well of a tube strip at **room temperature**
- If not already, using a **wide-bore** tip, dispense **15 µl** 1 ng/µl HMW gDNA into each well of a tube strip at **room temperature**
- Using a **narrow-bore** tip, transfer **10 µl** diluted HMW gDNA solution into pre-aliquoted 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)	24 rxns + 10% xs (µl)
Genome Reagent Mix	89.5	2363
Additive A	3	79
Genome Enzyme Mix	5	132
Total	**97.5**	**2574**

Chromium™ Genome HT v2

2. GEM Generation & Barcoding

2.3 Loading the Chromium™ Genome Chip

- If <8 samples, dispense 50% glycerol solution to each unused well: 90 µl - row labeled 1, 85 µl - row labeled 2, 270 µl - row labeled 3. Do not add 50% glycerol solution to Recovery Wells (row labeled ◀)
- Using a **narrow-bore** tip, slowly dispense **90 µl** Sample Master Mix-denatured gDNA mix into bottom of row labeled **1**
- After centrifuging and pipette mixing the Gel Bead Plate, 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 (~20 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

Chromium™ Genome HT v2

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

Obtain:

- Recovery Agent
- Qiagen Buffer EB
- Bio-Rad 10% Tween 20
- 10x™ Magnetic Separator

- *Ensure no precipitate in liquid reagents before use*

Prepare:

- 80% Ethanol (10 ml for 8 samples)

Thaw at 65°C:

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

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 from bottom of tube, 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 **51 µl Elution Solution I**
- Incubate **30 sec**. Resuspend pellet in solution
- Incubate **5 min**
- Centrifuge briefly. Magnet **L**
- Transfer **50 µl** sample to new tube strip

DynaBeads Cleanup Mix	1X (µl)	24 rxns + 10% xs (µl)
Buffer Sample Clean Up 1	136	3590
DynaBeads MyOne Silane	8	211
Additive A	6	159
Total	**150**	**3960**

Elution Solution I	1 rxn (µl)	24 rxns + 25% xs (µl)
Buffer EB	89	2670
10% Tween 20	1	30
Additive A	10	300
Total	**100**	**3000**

3.2 Post GEM Incubation Cleanup - SPRIselect

- 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
- Store at 4°C ≤72 h or at –20°C ≤2 weeks

Elution Solution II	1 rxn (µl)	24 rxns + 25% xs (µl)
Buffer EB	98	2490
● Additive A	2	60
Total	**100**	**3000**

3.3 Post GEM QC

- Run 1 µl 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
- Chromium™ i7 Index Plate
- Beckman Coulter SPRIselect Reagent
- Agilent Bioanalyzer DNA 1000 Kit, or
- Agilent TapeStation D1000 ScreenTape & Reagents

- *Ensure no precipitate in liquid reagents before use*

Place on ice:

- End Repair and A-tailing Enzyme
- DNA Ligase
- Amplification Master Mix
- Kapa DNA Quantification Kit for Illumina Platforms

Obtain:

- Qiagen Buffer EB
- 10x™ Magnetic Separator

Prepare:

- 80% Ethanol (10 ml for 8 samples)

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 End Repair & A-tailing

End Repair and A-tailing Mix	1X (µl)	24 rxns + 10% xs (µl)
Nuclease-Free Water	2.5	66
End Repair & A-tailing Buffer	7.5	198
End Repair & A-tailing Enzyme	15	396
Total	**25**	**660**

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

- Add **25 µl** End Repair and A-tailing Mix to each tube containing 50 µl sample from Post GEM Incubation Cleanup
- Pipette mix thoroughly and centrifuge briefly
- Place tube strip in the thermal cycler and run the End Repair and A-tailing program (right)

4.2 Adaptor Ligation

Adaptor Ligation Mix	1X (µl)	24 rxns + 10% xs (µl)
Ligation Buffer	22	580
DNA Ligase	11	290
Adaptor Mix	2.5	66
Total	**35.5**	**936**

- Add **35.5 µl** Adaptor Ligation Mix to each tube containing 75 µl sample from the End Repair and A-tailing step
- Pipette mix thoroughly and centrifuge briefly

Lid Temperature	Reaction Volume	Run Time
30°C	110 µl	15 min

Step	Temperature	Time
1	20°C	15:00

- Place tube strip in the thermal cycler and run the Ligation program (above)

4.3 Post Ligation Cleanup - SPRIselect

- 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

4.4 Sample Index PCR

Sample Index PCR Mix	1X (µl)	24 rxns + 10% xs (µl)
Amplification Master Mix	50	1320
Forward PCR Primer	5	132
Total	**55**	**1452**

- Add **55 µl** Sample Index PCR Mix into each tube containing 40 µl purified Post Ligation sample
- Add **5 µl** of a single Chromium™ i7 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)

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~30 min

Step	Temperature	Time
1	98°C	0:45
2	98°C	0:20
3	54°C	0:30
4	72°C	0:20
5	Go to Step 2, 9X (for 10 cycles total)	
6	72°C	1:00
7	4°C	Hold

- Store at 4°C ≤72 h or proceed to next step

4.5 Post Sample Index Double Sided Size Selection - SPRIselect

- Transfer **96 µl** of the indexed PCR samples to a new tube strip. If the sample is less than 96 µl, bring the total volume to 96 µl with Buffer EB.
- Add **48 µl** SPRIselect Reagent
- Mix. Incubate **5 min**
- Magnet **H**
- **DO NOT discard supernatant**
- **Transfer 135 µl supernatant to new tube strip**
- Discard old tube strip
- Add **18 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

4.6 Post Library Construction QC

- Either run 1 µl sample on the Agilent Bioanalyzer DNA 1000 chip to determine fragment size
- Or run 1 µl sample on the Agilent TapeStation D1000 ScreenTape to determine fragment size

4.7 Post Library Construction Quantification

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

5. Sequencing

– The Genome Solution produces Illumina-ready sequencing libraries. See the Chromium™ Genome Reagent Kits v2 User Guide (Document CG00043, Section 5) for further information

Sequencing Depth Recommendations

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

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

Sequencing Run Parameters

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

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

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

Changing the Definition of Sequencing™

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