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Chromium™

# Genome Reagent Kits v2

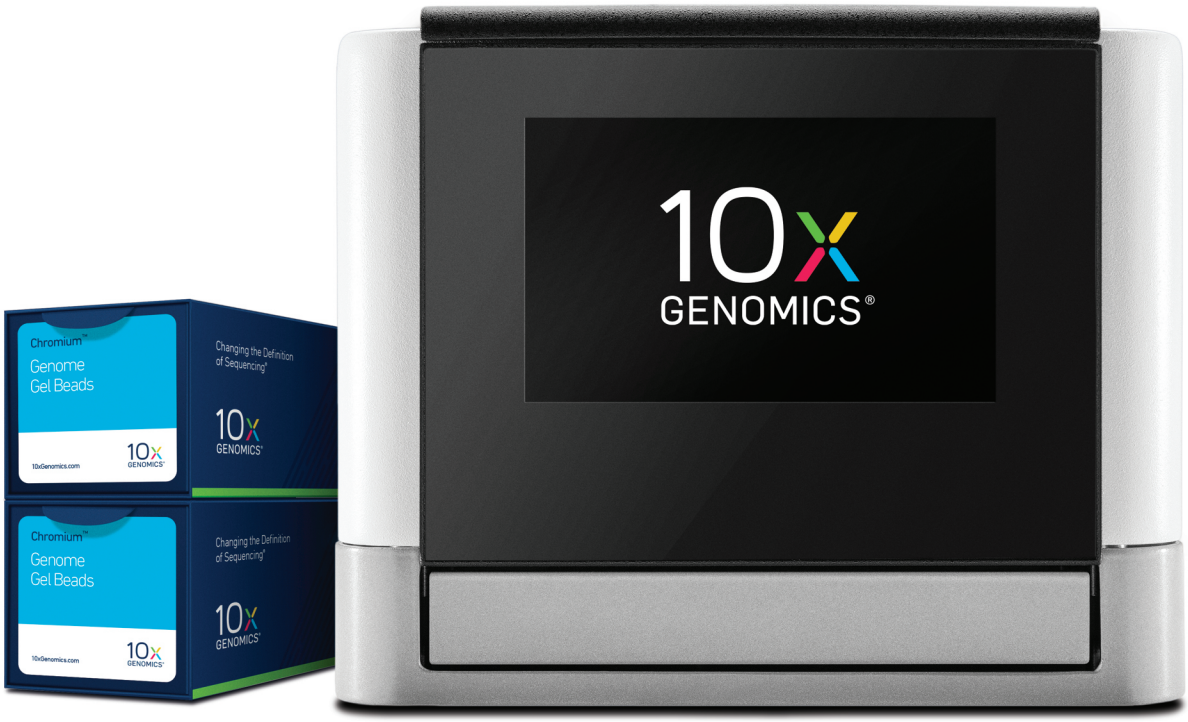
## *Quick Reference Cards*

FOR USE WITH

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

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 

# Chromium™ Genome v2

## 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:**

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

**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) | 8.8X (µl) |
|---|---|---|
| Genome Reagent Mix | 89.5 | 788 |
| Additive A | 3 | 26 |
| Genome Enzyme Mix | 5 | 44 |
| **Total** | **97.5** | **858** |

# Chromium™ Genome 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 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 (~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 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) | 8.8X (µl) |
|---|---|---|
| ● Buffer Sample Clean Up 1 | 136 | 1197 |
| DynaBeads MyOne Silane | 8 | 70 |
| ● 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

- 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) | 10 rxns (µl) |
|---|---|---|
| Buffer EB | 98 | 980 |
| ● Additive A | 2 | 20 |
| **Total** | **100** | **1000** |

## 3.3 Post GEM QC

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

# Chromium™ Genome v2

## 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) | 8.8X (µl) |
|---|---|---|
| Nuclease-Free Water | 2.5 | 22 |
| End Repair & A-tailing Buffer | 7.5 | 66 |
| End Repair & A-tailing Enzyme | 15 | 132 |
| **Total** | **25** | **220** |

| 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)

# Chromium™ Genome v2

## 4. Library Construction

## 4.2 Adaptor Ligation

| Adaptor Ligation Mix | 1X (µl) | 8.8X (µl) |
|---|---|---|
| Ligation Buffer | 22 | 194 |
| DNA Ligase | 11 | 97 |
| Adaptor Mix | 2.5 | 22 |
| **Total** | **35.5** | **313** |

- 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) | 8.8X (µl) |
|---|---|---|
| Amplification Master Mix | 50 | 440 |
| Forward PCR Primer | 5 | 44 |
| **Total** | **55** | **484** |

- 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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