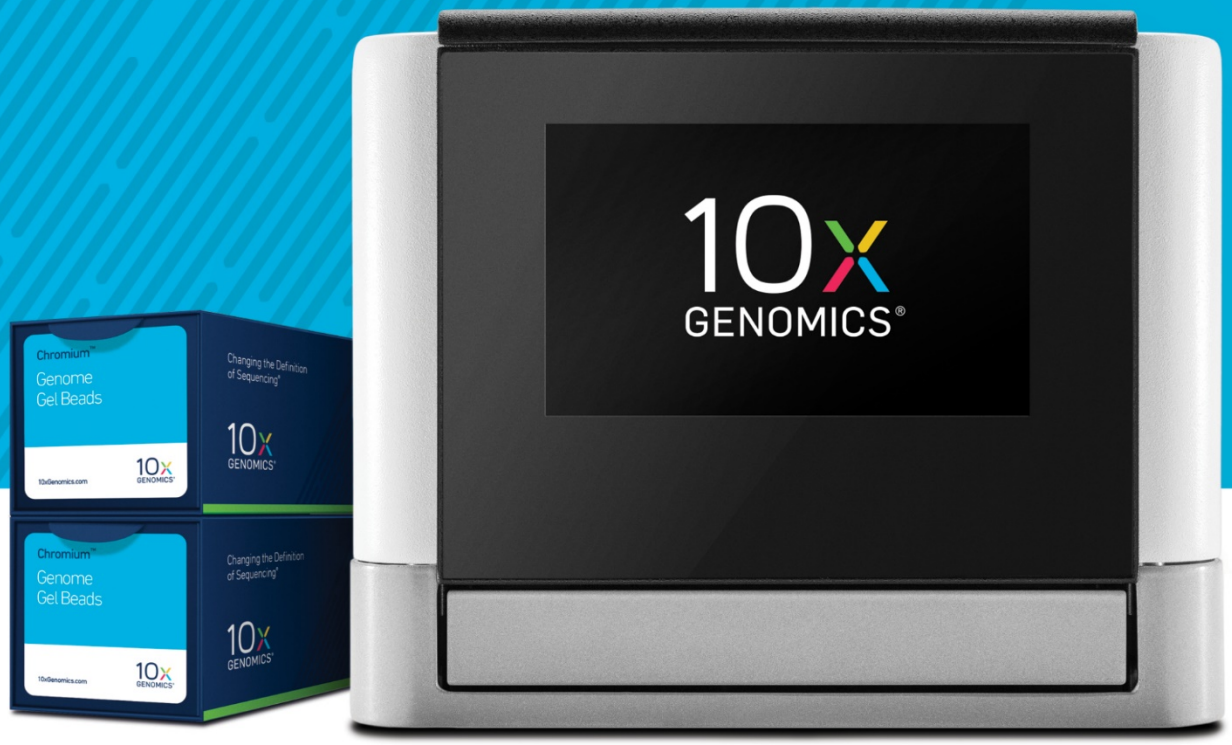
Chromium™

# Genome Reagent Kits User Guide

**FOR USE WITH**

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

**Chromium™ Genome Chip Kit PN-120216**




10x GENOMICS®

10xGenomics.com

# Notices

## Manual Part Number

CG00022 Rev C

## Legal Notices



## Customer Information and Feedback

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

Email: support@10xgenomics.com

10x Genomics

7068 Koll Center Parkway

Suite 401

Pleasanton, CA 94566 USA

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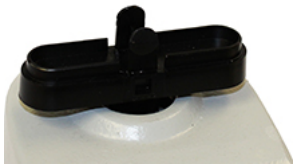
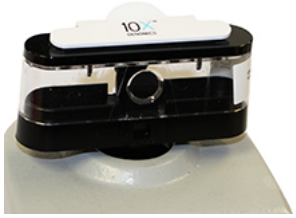
# Chromium™ Genome Reagent Kits – Components

| Product | Description | # | Part Number |
|---|---|---|---|
| Chromium™ Genome Library Kit (store at –20°C) | | | 120215 |
| Reagents Module 1 | Denaturing Agent | 1 | 220072 |
| | Genome Enzyme Mix | 1 | 220059 |
| | Genome Reagent Mix | 1 | 220060 |
| | Additive A | 1 | 220074 |
| | Control DNA | 1 | 220045 |
| | Surrogate Fluid | 1 | 220021 |
| | Buffer for Sample Cleanup 1 | 2 | 220020 |
| | | | |
| Reagents Module 2 | End Repair and A-tailing Buffer | 1 | 220046 |
| | End Repair and A-tailing Enzyme | 1 | 220047 |
| | Ligation Buffer | 1 | 220048 |
| | DNA Ligase | 1 | 220049 |
| | PCR Master Mix | 1 | 220024 |
| | Adaptor Mix | 1 | 220026 |
| | Forward PCR Primer | 1 | 220061 |
| | Post Capture PCR Master Mix | 1 | 220036 |
| | Post Capture PCR Primers | 1 | 220037 |
| | | | |
| | Genome Sample Index Plate | 1 | 210134 |
| Chromium™ Genome Gel Bead Kit (store at –80°C) | | | 120214 |
| | Genome Gel Beads | 2 | 220058 |
| Chromium™ Genome Chip Kit (store at ambient temperature) | | | 120216 |
| | Genome Chips | 6 | 230007 |
| | Gaskets | 6 | 370017 |
| | Partitioning Oil | 4 | 220017 |
| | Recovery Agent | 6 | 220016 |

# 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.<br>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.<br>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.<br>   | 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

| Product | Description | Part Number (US) |
|---|---|---|
| Qiagen | Buffer EB | 19086 |
| Thermo Fisher | DynaBeads® MyOne™ Silane Beads* | 37002D |
| | Nuclease-Free Water | AM9937 |
| | Qubit® dsDNA HS Assay Kit | Q32854 |
| | Qubit® Assay tubes | Q32856 |
| | Qubit 3.0 Fluorometer | Q33216 |
| Sigma | Ethanol, Pure (200 Proof, anhydrous) | 459836-500ML |
| Beckman Coulter | SPRIselect Reagent Kit* | B23318 |
| USA Scientific | TempAssure PCR 8-tube strip* (alternate to Eppendorf) | 14024700 |
| Eppendorf | twin.tec® 96-Well PCR Plate* Semi-skirted§ | 951020362 |
| | twin.tec® 96-Well PCR Plate* Divisible, unskirted§ | 0030133374 |
| | twin.tec® 96-Well PCR Plate* Unskirted§ | 0030133390 |
| | ThermoMixer C®** | 5382000015 |
| | SmartBlock™ 1.5 mL, thermoblock for 24 Reaction Vessels | 5360000038 |
| | SmartBlock™ PCR 96, thermoblock for PCR plates 96 | 5306000006 |
| | DNA LoBind Tube Microcentrifuge Tube, 1.5 mL* | 022431021 |
| | DNA LoBind Tube Microcentrifuge Tube, 2.0 mL* | 022431048 |
| | PCR Tubes 0.2 mL 8-tube strips* (alternate to USA Scientific) | 951010022 |
| Bio-Rad | PX1 PCR Plate Sealer** | 1814000 |
| | Optical Flat 8-Cap Strips | TCS0803 |
| | Pierceable Foil Heat Seal** | 1814040 |
| | 10% Tween 20 | 1610781 |
| | Hard-Shell Low-Profile Thin-Wall 96-Well Skirted PCR Plates** | HSP9665 |
| | Microseal 'B' Adhesive Seals | MSB1001 |
| KAPA Biosystems | Illumina Library Quantification Kit | KK4824 |
| - | qPCR Instrument and compatible consumables | - |
| VWR | Vortex Mixer* | 10153-838 |
| | Divided Polystyrene Reservoirs** | 41428-958 |
| 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 |
| Covaris | M220 Focused Ultrasonicator™*** | 500295 |
| | M220 Holder microTUBE*** | 500301 |
| | microTUBE AFA Fiber Screw-Cap 6 x 16 mm*** | 520096 |

| Product | Description | Part Number (US) |
|---|---|---|
| Ricca Chemical Company | Glycerin (glycerol), 50% (v/v) Aqueous Solution (additional Surrogate Fluid, if needed) | 3290-32 |
| Integrated DNA Technologies | xGen® Universal Blocking Oligo – TS-p5<br>xGen® Universal Blocking Oligo – TS-p7(8nt) | 1016184<br>1016188 |
| Rainin | Tips LTS 20UL Filter RT-L10FLR | 17007957 |
| | Tips LTS 200UL Filter RT-L200FLR | 17007961 |
| | Tips LTS 1ML Filter RT-L1000FLR | 17007954 |
| | Tips LTS W-O 200UL Fltr RT-L200WFLR*† | 17014294 |
| | Tips LTS W-O 1MLUL Fltr RT-L1000WFLR*† | 17014297 |
| | Pipet-Lite LTS Pipette L-2XLS+ | 17014393 |
| | 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 |
| | Pipet-Lite Multi Pipette L8-50XLS+ | 17013804 |
| | Pipet-Lite Multi Pipette L8-200XLS+ | 17013805 |

*No substitutions are allowed. Items have been validated by 10x Genomics® and are required for Genome workflow, training and system operations. § Eppendorf twin.tec® brand PCR plates are required to ensure stability of GEM emulsions, but the specific model should be selected based on compatibility with thermal cycler in use. **Substituting materials may adversely affect system performance and are not supported. ‡Either Bioanalyzer or TapeStation needed for quality control. ***Models LE220 and S2 have also been validated for use with the manufacturer's recommended tubes. †Wide orifice tips are required for HMW gDNA handling.

### PCR 8-tube strips

USA Scientific TempAssure PCR 8-tube strip and Eppendorf PCR Tubes 0.2 mL 8-tube strips have been validated by 10x Genomics®.

If USA Scientific or Eppendorf 8-tube strips are not available in your region, alternatives are MicroAmp® and BIOplastics 8-tube strips and caps.

## Recommended Thermal Cyclers

Thermal cyclers used with the Genome Protocol must support uniform heating of 100 µl emulsion volumes. Thermal cyclers recommended for use with the Genome Protocol are:

- Bio-Rad C1000 Touch™ Thermal Cycler with 96-Deep Well Reaction Module (# 1851197)
- Eppendorf MasterCycler® Pro (# North America 950030010, International 6321 000.019)
- Thermo Fisher Veriti© 96-Well Thermal Cycler (# 4375786)

# The Genome Reagent Kit Protocol

Stepwise Objectives

Steps & Timing

# The Genome Reagent Kit Protocol – Stepwise Objectives

## Step 1 – HMW gDNA Extraction

The Chromium™ Genome Protocol generates long-range information across the length of individual DNA molecules, which is used for both the Chromium Genome and Exome Solutions. For both of these solutions, starting the process with high molecular weight (HMW) genomic DNA (gDNA) results in better performance, such as increased haplotype phase block length and ability to call structural variants. Optimal performance has been characterized on input gDNA with a mean length greater than 50 kb, and this protocol outlines the extraction of HMW gDNA with optimal size from live cells.

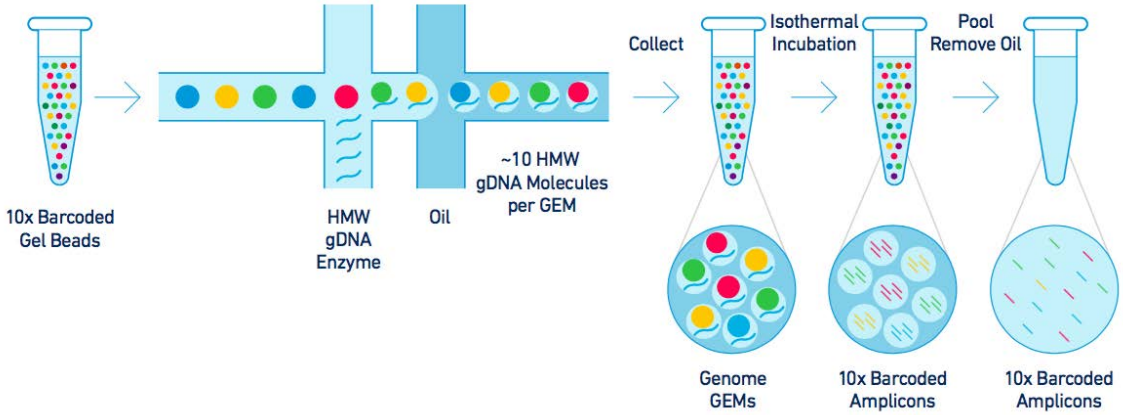
## Step 2 – GEM Generation & Barcoding

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

Approximately 1-1.2 ng (~ 300-360 haploid genome equivalents) of template gDNA is partitioned across over1 million GEMs and consequently only a small number of genomic equivalents are loaded in each GEM (~500 kb after accounting for system losses, or 0.02% of the haploid human genome). With such dispersion of template, it is highly unlikely that two distinct gDNA molecules that cover the same locus with opposing haplotypes will have the same 10x Barcode. Loading the correct amount of gDNA into the system during GEM generation is critical to optimal performance. It is also important to note only approximately 50% of loaded gDNA is incorporated into the final sequencing library – the remainder is left behind in the microfluidic Genome Chip.

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

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




## Step 3 – Post GEM Cleanup & QC

Silane magnetic beads are used to remove leftover biochemical reagents from the post GEM reaction mixture. Solid Phase Reversible Immobilization (SPRI) beads are used to optimize the appropriate DNA size

range for library preparation. Importantly, there are subtle differences when assaying the whole genome and the exome called out in the protocols.

## Step 4 – Library Construction

When assaying the exome, an additional shearing step further optimizes the 10x™ Barcoded fragment size prior to library construction. Read 1 sequence and the 10x Barcode are added to the molecules during the GEM incubation. P5 and P7 primers, Read 2, and Sample Index are added during library construction via end repair, A-tailing, adaptor ligation, and amplification. The final libraries contain the P5 and P7 primers used in Illumina bridge amplification.

When assaying the whole genome, these libraries are ready for sequencing (Step 7). When assaying the exome, these libraries are ready for target enrichment through hybridization (Step 5) and capture (Step 6).

## Step 5 – Target Enrichment (Hybridization) – Exome Protocol Only

When assaying the exome, the sample is enriched using a modified two-step process with Agilent SureSelectXT Target Enrichment System for Illumina Paired-End Sequencing. First is hybridization with biotinylated SureSelect capture baits.

Target enrichment enables access to low complexity and duplicated regions, megabase-scale phasing, structural and copy number variation, while improving SNP calls.

## Step 6 – Target Enrichment (Capture) – Exome Protocol Only

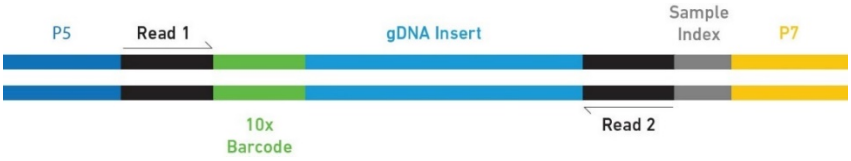
When assaying the exome, the second enrichment step is capturing the hybridized biotinylated SureSelect baits onto Streptavidin beads extracts exome sequences for subsequent sequencing.

## Step 7 – Sequencing Libraries

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

Both Genome and 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.

Final Library Construction:

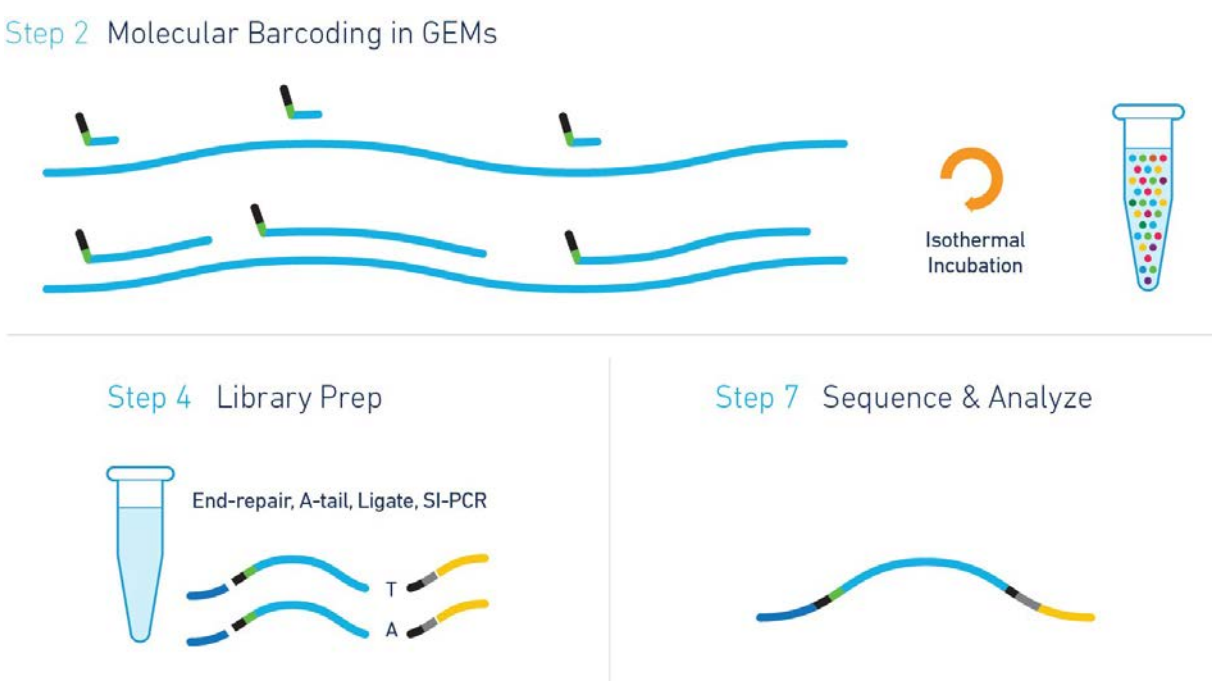



## Library Analysis – Chromium™ Software

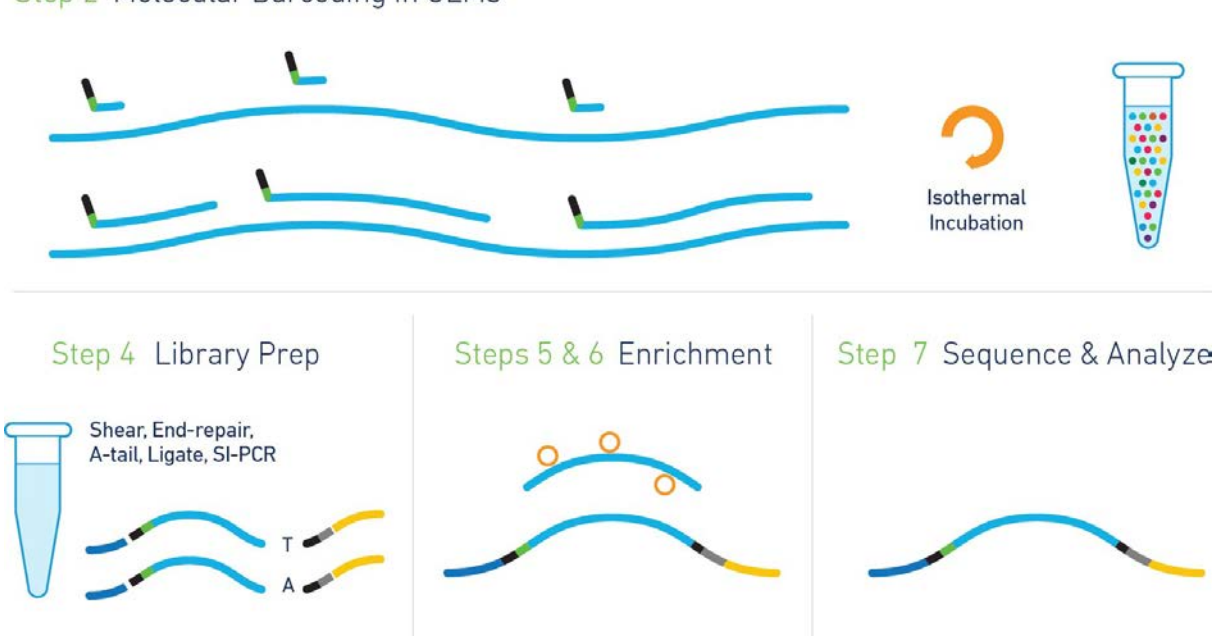
Built upon widely accepted aligners and variant callers, the Long Ranger™ analysis pipelines leverage the Linked-Reads created by 10x Barcodes to yield insight into megabase-scale haplotype phasing, structural variant detection and phasing, and greater accuracy in SNP and indel calling. The Supernova™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

## Protocols Steps – Genome and Exome Protocols at a Glance

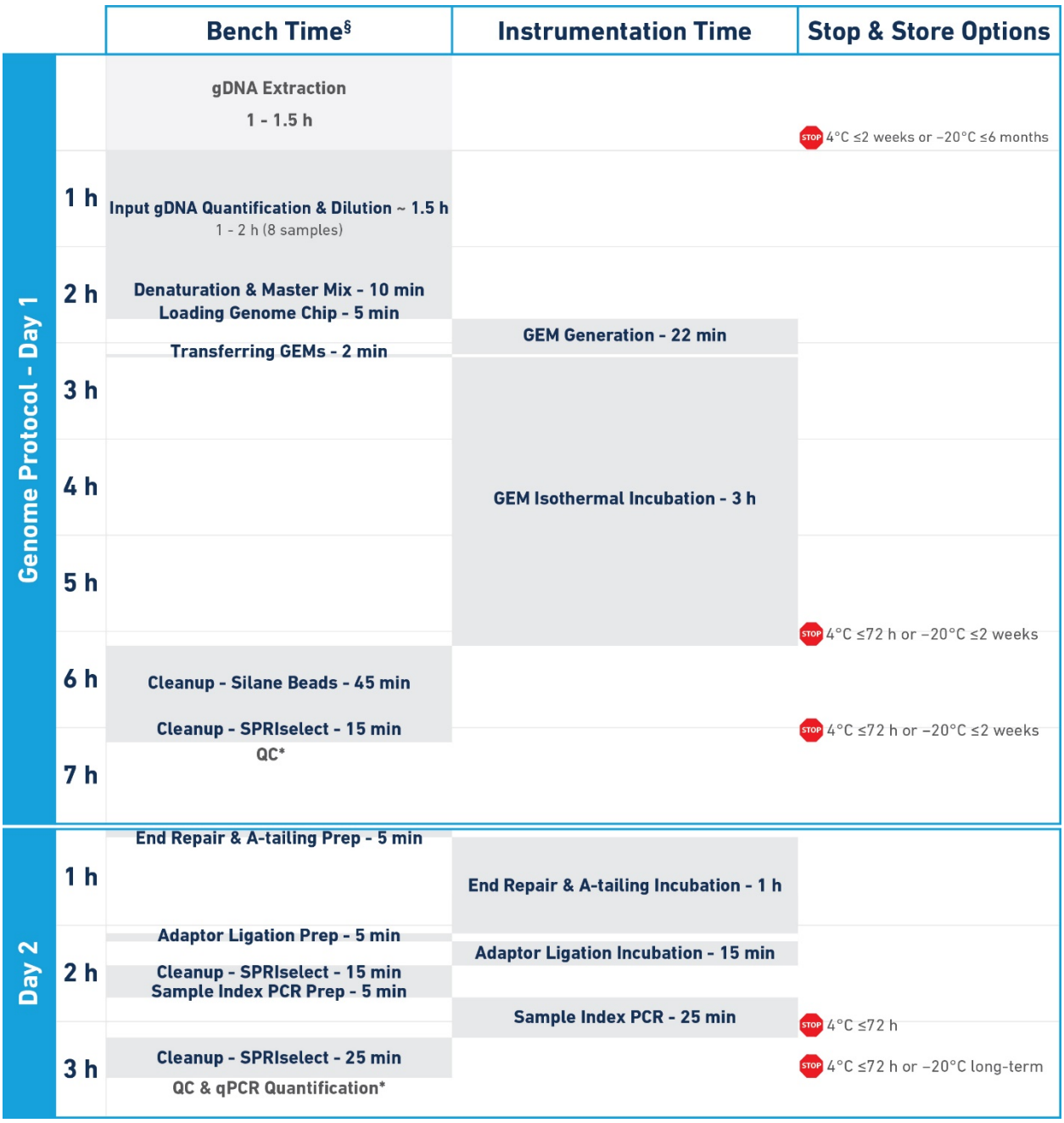
### Genome Protocol:




### Exome Protocol:



# The Genome Protocol – Steps & Timing




*§Estimates including hands-on & benchtop incubation times for processing 8 samples.*
**Bioanalyzer/TapeStation QC & qPCR Quantification times not included.*

# The Exome Protocol – Steps & Timing

| | | Bench Time§ | Instrumentation Time | Stop & Store Options |
|---|---|---|---|---|
| Exome Protocol - Day 1 | | gDNA Extraction<br>1 - 1.5 h | | STOP 4°C ≤2 weeks or –20°C ≤6 months |
| | 1 h | Input gDNA Quantification & Dilution ~ 1.5 h<br>1 - 2 h (8 samples) | | |
| | 2 h | Denaturation & Master Mix - 10 min<br>Loading Genome Chip - 5 min | | |
| | | Transferring GEMs - 2 min | GEM Generation - 22 min | |
| | 3 h | | GEM Isothermal Incubation - 3 h | |
| | 4 h | | | |
| | 5 h | | | STOP 4°C ≤72 h or –20°C ≤2 weeks |
| | 6 h | Cleanup - Silane Beads - 45 min<br>Cleanup 1 - SPRIselect - 15 min | | |
| | 7 h | Cleanup 2 - SPRIselect - 15 min<br>QC* | | STOP 4°C ≤72 h or –20°C ≤2 weeks |
| Day 2 | 1 h | Shearing** - 35 min<br>End Repair & A-tailing Prep - 5 min | | |
| | 2 h | | End Repair & A-tailing Incubation - 1 h | |
| | | Adaptor Ligation Prep - 5 min<br>Cleanup - SPRIselect - 15 min | Adaptor Ligation Incubation - 15 min | |
| | 3 h | Sample Index PCR Prep - 5 min | Sample Index PCR - 25 min | STOP 4°C ≤72 h<br>STOP 4°C ≤72 h or –20°C long-term |
| | | Cleanup - SPRIselect - 15 min<br>QC Prep - 5 min | | |
| | 4 h | Dry-down Prep - 5 min | QC - 45 min | |
| | 5 h | | Library Dry-down - 1.5 h | |
| | 6 h | Enrichment (Hybridization) Prep - 1 h | | |
| | 7 h | | Hybridization - 16 - 24 h (overnight) | |
| Day 3 | 1 h | Capture Reagent Prep - 10 min<br>Enrichment (Capture) - 1.5 h | | |
| | 2 h | Post Capture PCR Prep - 5 min | Post Capture PCR - 20 min | |
| | | Cleanup - SPRIselect - 15 min | | STOP 4°C ≤72 h or –20°C long-term |
| | 3 h | QC & qPCR Quantification* | | |

*§Estimates including hands-on & benchtop incubation times for processing 8 samples.*
**Bioanalyzer/TapeStation QC & qPCR Quantification times not included. ** Shearing time on a Covaris M220 instrument.*

# Protocol Step 1

# HMW gDNA Extraction

Extracting high molecular weight genomic DNA to maximize Linked-Reads

1

# 1. HMW gDNA Extraction

## Tips

### High Input DNA Length Results in Optimal Performance

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

### Best Practices for Handling HMW gDNA

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

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

## Getting Started!

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

# HMW gDNA Extraction

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

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

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

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

*NOTE*

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

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

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

*NOTE*

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

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

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

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

*Repeat*

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

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

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

*Repeat*

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

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

*CRITICAL!*

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

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

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

*Repeat*

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

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

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

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


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

# Protocol Step 2

# GEM Generation & Barcoding

Partition template HMW gDNA across millions of GEMs for barcoding

2

# 2. GEM Generation & Barcoding

## Tips

### Importance of Emulsion-safe Plastic Consumables

Some plastics can interact with and destabilize GEMs. It is therefore critical to use validated emulsion-safe plastic consumables when handling GEMs. 10x Genomics® has validated Eppendorf twin.tec® PCR plates and Rainin LTS Low retention pipette tips as GEM-compatible plastics. Substituting these materials can adversely affect performance.

### Importance of DNA Loading Quantity

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

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

### High Sensitivity DNA Quantification

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

### Best Practices for HMW gDNA Handling

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

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

### Best Practices for Reagent Handling

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

- Ensuring the reagents are fully thawed and thoroughly mixed before use. Keep all enzyme components and master mixes on ice during setup and promptly move reagents back to the recommended storage temperature when possible.
- Equilibrate the Genome Gel Bead Strip to room temperature for >30 min before use and store any unused Genome Gel Beads at –80°C. Avoid more than 4 freeze-thaw cycles at –80°C and never store at –20°C.

## Best Practices for GEM Generation

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

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

## Prepare Surrogate Fluid if Processing Fewer than 8 Reactions

It is critical all unused wells in Rows labeled 1, 2 and 3 of the Genome contain Surrogate Fluid prior to running the Chromium Controller. Surrogate Fluid for up to 4 Genome channels is provided in the Library Kit. See Practical Tips & Troubleshooting (Section 8) for information on purchasing or generating more Surrogate Fluid, if required.

## Additional Practical Tips & Troubleshooting

Further information can be found in Practical Tips & Troubleshooting (Section 8):

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

# Getting Started!

## Equilibrate to room temperature before use:

| Item | Storage Location |
|---|---|
| Qubit® HS standards | Follow manufacturer's instructions |
| Genome Gel Beads (equilibrate to room temperature 30 min before loading the Genome Chip) | **–80°C** |
| Additive A (vortex, verify no precipitate, centrifuge briefly) | –20°C |
| Denaturing Agent (vortex, centrifuge briefly) | –20°C |
| Heat block from plate sealer | - |

## Surrogate Fluid

- If processing fewer than 8 reactions, equilibrate Surrogate Fluid to room temperature. See Practical Tips & Troubleshooting (Section 8) for information on purchasing or generating more Surrogate Fluid, if required.

## Place on ice:

| Item | Storage Location |
|---|---|
| Genome Reagent Mix (thaw, vortex, centrifuge briefly) | –20°C |
| Genome Enzyme Mix (flick gently downwards, centrifuge briefly | –20°C |
| Chilled metal block resting on ice | - |

## Obtain:

| Item | Storage Location |
|---|---|
| Partitioning Oil | Ambient temperature |
| Qiagen Buffer EB | Follow manufacturer's instructions |
| Genome Chip(s), 10x™ Gasket(s) and 10x Chip Holder | Ambient temperature |

## 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, 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 instructions 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) Depending on whether assaying the whole genome or the exome, dilute the gDNA solution to the concentration indicated below in Buffer EB in an 8-tube strip. Gently mix 10 times with a multi-channel pipette and wide-bore pipette tips.

| Genome Protocol | Exome Protocol |
|---|---|
| 1 ng/µl | 1.2 ng/µl |

*Ideally prepare 50 µl of the final concentration of 1 ng/µl (Genome Protocol) or 1.2 ng/µl (Exome Protocol). 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 DNA 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 DNA solution are within the ranges shown below before proceeding to Preparing GEM Reagent Mix. If recorded concentrations are out of range, repeat from step d.

| Genome Protocol – Acceptable Range | Exome Protocol – Acceptable Range |
|---|---|

| | |
|---|---|
| 0.8 – 1.2 ng/µl | 1.0 – 1.4 ng/µl |

## 2.2. Preparing Sample Master Mix

**NOTE**

*If processing up to 4 Chromium™ Genome Chips, prepare all Sample Master Mix combined with Denatured DNA (Steps 2.2a – 2.2j) before proceeding with Step 2.3.*

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

**NOTE**

*Volumes for 8 reactions are listed in all reagent tables and include 10% excess (i.e. 8.8X).*

| 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** |

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) Transfer the 1-1.2 ng/µl HMW gDNA solution from step 2.1 into a new tube strip using wide-bore pipette tips if the diluted gDNA is not already in a tube strip at room temperature.

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 2.5 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(s) and return to the chilled block.

## 2.3. Loading the Genome Chip

a) Place a Genome Chip in a 10x™ Chip Holder. See Practical Tips & Troubleshooting (Section 8) 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 Surrogate Fluid to each unused well. Add only Surrogate Fluid (not Partitioning Oil) 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 Surrogate Fluid to Recovery Wells (row labeled ◀)*

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**. Inspect for bubbles at the bottom of the wells. If found, remove carefully with a pipette tip.


d) Snap the Genome Gel Bead Strip into a 10x Vortex Adaptor. Vortex for 15 sec.

e) 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 well levels look even.

**NOTE**

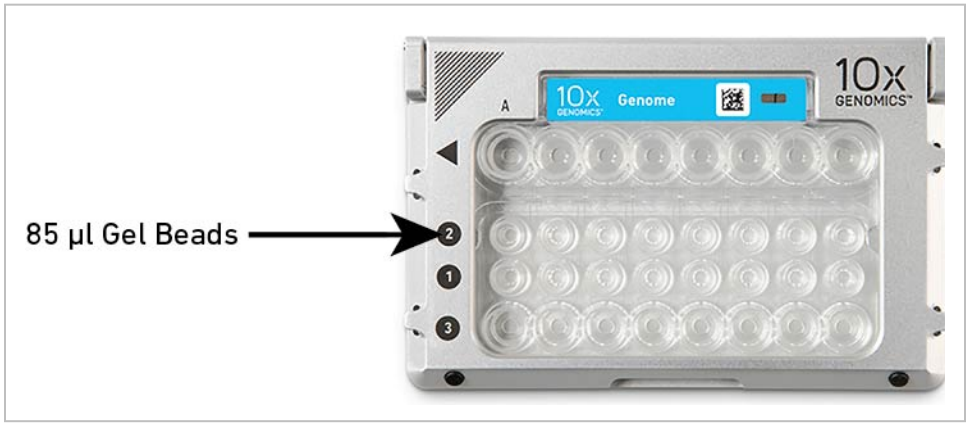
*Pipette Genome Gel Beads slowly as they have a viscosity similar to high-concentration glycerol.*

f) Carefully puncture the foil seal and slowly aspirate 85 µl Genome Gel Beads.

**NOTE**

*If processing fewer than 8 samples, only puncture sufficient wells in the Genome Gel Bead Strip.*

g) Slowly dispense the Genome Gel Beads **into the bottom of wells in the row labeled 2**, taking care not to introduce bubbles. Inspect for bubbles at the bottom of the wells. If found, remove carefully with a pipette tip.

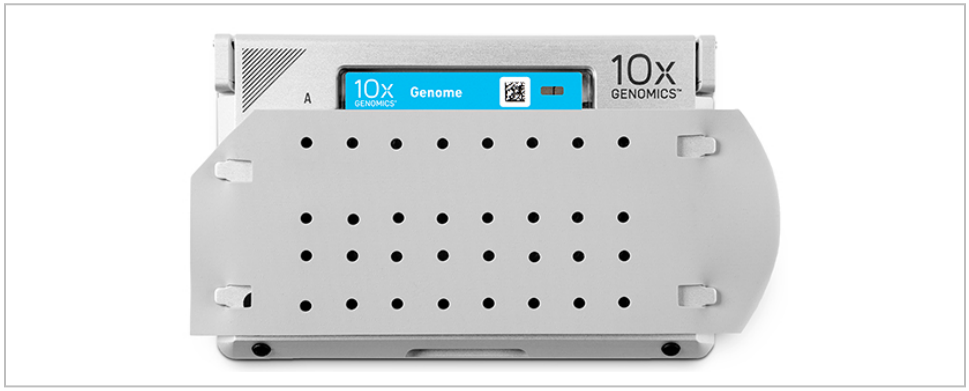



h) Pipette 270 µl Partitioning Oil into the wells in the **row labeled 3**. Pipette Partitioning Oil into wells one at a time. Do not use a reagent reservoir for the Partitioning Oil.


***CRITICAL!***

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

i) 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.



## 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 (~22 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.

c) Open the 10x Chip Holder and fold the lid back until it clicks to expose the wells at a 45-degree angle.

d) Check for volume uniformity in the Gel Bead, Sample, and Partitioning Oil wells remaining in the Genome Chip.

***NOTE***

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

e) Slowly aspirate 125 µl GEMs from the lowest points of the Recovery Wells (row labeled ◀) without creating a seal between the tips and the bottom of the wells. Avoid introducing air bubbles.

**NOTE** *Pipette GEMs slowly as they have a high viscosity.*

f) Withdraw pipette tips from the wells and verify that there is no air in the tips. GEMs should appear opaque and uniform across all channels.

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

g) Slowly dispense the GEMs into 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. 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 DNA (prepared in Step 2.2) and diluted DNA 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. The run will take ~3 h 10 min.

NOTE

*A reaction volume of 125 µl is the preferred setting on the Bio-Rad C1000 Touch™ Thermal Cycler. If using an alternate thermal cycler, the highest reaction volume setting should be used.*

| Lid Temperature | Reaction Volume | |
|---|---|---|
| 75°C | 125 µl | |
| | | |
| **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 2 weeks before proceeding to Post GEM Incubation Cleanup.

# Protocol Step 3

# Post GEM Incubation Cleanup & QC

Isolate and size DNA for library construction

3

# 3. Post GEM Incubation Cleanup & QC

## Tips

### Best Practices

Ensure that the reagents are fully thawed and thoroughly mixed before use. During the bead-based cleanup steps, ensure that the samples are thoroughly mixed with the Silane beads or the SPRIselect Reagent to achieve optimal recovery.

### Best Practices – Post GEM QC

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

# Getting Started!

## Equilibrate to room temperature before use:

| Item | Storage Location |
|---|---|
| Additive A (vortex, verify no precipitate, centrifuge briefly) | –20°C |
| DynaBeads® MyOne™ Silane beads | Follow manufacturer's instructions |
| Beckman Coulter SPRIselect Reagent | Follow manufacturer's instructions |
| Agilent Bioanalyzer High Sensitivity Kit | Follow manufacturer's instructions |

## Obtain:

| Item | Storage Location |
|---|---|
| Recovery Agent | Ambient temperature |
| Qiagen Buffer EB | Follow manufacturer's instructions |
| Bio-Rad 10% Tween 20 | Follow manufacturer's instructions |

## Thaw at 65°C:

- Thaw Buffer for Sample Cleanup 1 for 10 min at 65°C at max speed on a thermomixer. Verify there are no visible crystals. Let cool to room temperature.

## Prepare 80% Ethanol (10 ml for 8 Genome Protocol samples or 20 ml for 8 Exome Protocol 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.*

e) Vortex DynaBeads MyOne Silane beads until fully resuspended. Prepare the DynaBeads Cleanup Mix by adding reagents in the order shown below. Note different volumes for when assaying the whole genome or exome. Vortex mix thoroughly and use immediately.

| DynaBeads Cleanup Mix | Genome 1X (µl) | Genome 8.8X (µl) | Exome 1X (µl) | Exome 8.8X (µl) |
|---|---|---|---|---|
| Buffer for Sample Cleanup 1 | 140 | 1232 | 130 | 1144 |
| DynaBeads MyOne Silane | 4 | 35 | 14 | 123 |
| Additive A | 6 | 53 | 6 | 53 |
| **Total** | **150** | **1320** | **150** | **1320** |

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. Vortex mix thoroughly and centrifuge briefly. Aliquot 100 µl per reaction into each well of a tube strip.

| Elution Solution I | 1 reaction (µl) | 10 reactions (µl) |
|---|---|---|
| Buffer EB | 89 | 890 |
| 10% Tween 20 | 1 | 10 |
| Additive A | 10 | 100 |
| **Total** | **100** | **1000** |

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 50 µl Elution Solution I 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 the eluted sample to a new tube strip.

## 3.2. Post GEM Incubation Cleanup – SPRIselect

a) Depending on whether assaying the whole genome or the exome, prepare Elution Solution II by adding appropriate volume of reagents in the order shown below. Vortex mix thoroughly and centrifuge briefly.

| Elution Solution II | Genome 1 reaction (µl) | Genome 10 reactions (µl) | Exome 1 reaction (µl) | Exome 10 reactions (µl) |
|---|---|---|---|---|
| Buffer EB | 98 | 980 | 196 | 1960 |
| Additive A | 2 | 20 | 4 | 40 |
| **Total** | **100** | **1000** | **200** | **2000** |

b) Vortex the SPRIselect Reagent until fully resuspended. Depending on whether assaying the whole genome or the exome, add the appropriate volume of SPRIselect Reagent to each sample in the tube strip. Pipette mix thoroughly and centrifuge briefly.

| Genome Protocol | Exome Protocol |
|---|---|
| 35 µl (0.7X) | 60 µl (1.2X) |

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.

*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.

| Genome Protocol | Exome Protocol |
|---|---|
| SPRIselect cleanup is complete at this point, proceed to step n | Repeat steps b - m for an additional SPRIselect cleanup |

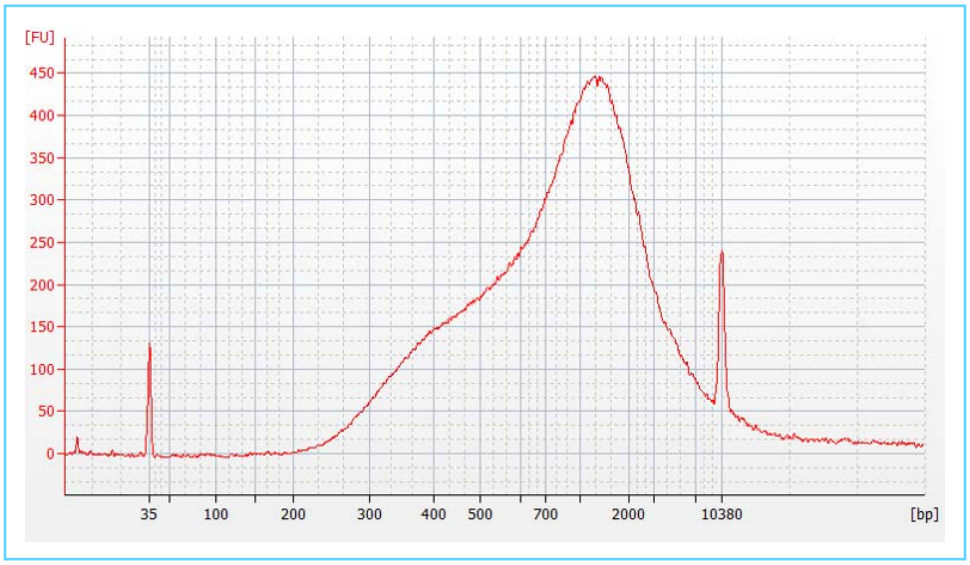
STOP

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

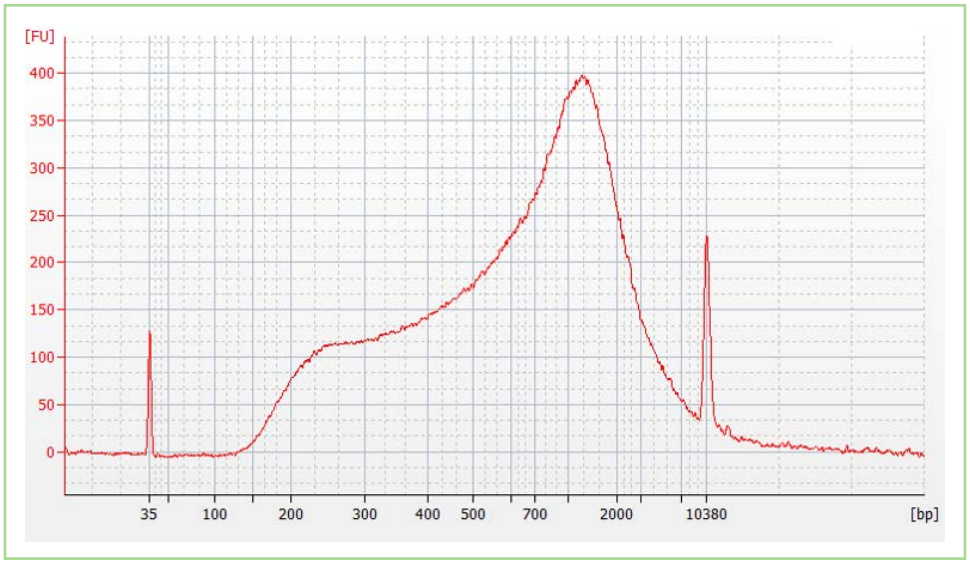
## 3.3. Post GEM QC

a) Run 1 µl Genome or Exome Protocol 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.

Genome Protocol Sample:




Exome Protocol Sample:



# Protocol Step 4

# Library Construction

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

# 4. Library Construction

## Tips

### General

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

### Best Practices – Reagents

Ensure that the reagents are fully thawed and thoroughly mixed before use. Keep all enzyme components and master mixes on ice during setup and promptly move back to the recommended storage temperature when possible.

### Best Practices – Shearing

Shearing the sample is only required when assaying the exome. Use Covaris's recommended settings for the available instrument to achieve a target peak size of 225 bp for a standard DNA sample.

### Best Practices – Sample Index PCR

Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run. Each sample index set is base-balanced to avoid monochromatic signal issues when it is the sole sample loaded on an Illumina sequencer.

### Best Practices – Post Library Construction QC

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

# Getting Started!

## Equilibrate to room temperature before use:

| Item | Storage Location |
|---|---|
| End Repair and A-tailing Buffer (vortex, verify no precipitate, centrifuge briefly) | –20°C |
| Forward PCR Primer | –20°C |
| Adaptor Mix | –20°C |
| Ligation Buffer (vortex, verify no clear pellet, centrifuge briefly) | –20°C |
| Genome Sample Index Plate | –20°C |
| Beckman Coulter SPRIselect Reagent | Follow manufacturer's instructions |
| Agilent Bioanalyzer DNA 1000 kit | Follow manufacturer's instructions |
| Agilent TapeStation D1000 ScreenTape and Reagents (if used for QC) | Follow manufacturer's instructions |

## Obtain:

| Item | Storage Location |
|---|---|
| Qiagen Buffer EB | Follow manufacturer's instructions |

## Place on ice:

| Item | Storage Location |
|---|---|
| End Repair and A-tailing Enzyme | –20°C |
| DNA Ligase | –20°C |
| PCR Master Mix | –20°C |
| Kapa DNA Quantification Kit for Illumina Platforms | Follow manufacturer's instructions |

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

# Library Construction

## 4.1. Shearing (Exome Protocol Only)

| Genome Protocol | Exome Protocol |
|---|---|
| Skip this step and proceed directly to step 4.2 | Proceed with this step 4.1 |

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. Library Construction: 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.

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

c) 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.

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

| Lid Temperature | Reaction Volume | |
|---|---|---|
| 85°C | 60 µl | |
| | | |
| **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. Library Construction: Adaptor Ligation

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

| **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** |

b) Add 50 µl Adaptor Ligation Mix to each tube containing 60 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** | |
|---|---|---|
| 30°C | 110 µl | |
| | | |
| **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. Depending on whether assaying the whole genome or the exome, add the appropriate volume of SPRIselect Reagent to each sample in the tube strip. Pipette mix thoroughly.

| Genome Protocol | Exome Protocol |
|---|---|
| 88 µl (0.8X) | 198 µl (1.8X) |

*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.

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

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 Genome Sample Index to each well and record their assignment. Pipette mix thoroughly and centrifuge briefly.

d) Depending on whether assaying the whole genome or the exome, amplify and index the library DNA in a thermal cycler for a total of 8 cycles or 12 cycles respectively. The run will take ~20-25 min.

| Lid Temperature | Reaction Volume | |
|---|---|---|
| 105°C | 100 µl | |
| | | |
| **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 in total) | |
| 5 – Exome Protocol | Go to step 2, 11X (for 12 cycles in total) | |
| 6 | 72°C | 1:00 |
| 7 | 4°C | Hold |


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

i. If assaying the whole genome, proceed to 4.6 Post Sample Index PCR SPRIselect.

ii. If assaying the exome, proceed to 4.7 Post Sample Index PCR SPRIselect.

## 4.6. Post Sample Index PCR Cleanup – SPRIselect – Genome Protocol

| Genome Protocol | Exome Protocol |
|---|---|
| Proceed with this step 4.6 | Skip this step and proceed directly to step 4.7 |

NOTEt

*This Genome Protocol step is a double-sided SPRIselect cleanup.*

a) Vortex the SPRIselect Reagent until fully resuspended. Add 50 µl (0.5X) SPRIselect Reagent to each sample in the tube strip. Pipette mix thoroughly.

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

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

CRITICAL!

*DO NOT discard supernatant.*

d) Transfer supernatant to a new tube strip and discard previous tube strip.

e) Vortex the SPRIselect Reagent until fully resuspended. Add 20 µl (0.7X final) SPRIselect Reagent to the supernatant in the new tube strip. Pipette mix thoroughly.

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

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

h) Carefully remove and discard the supernatant.

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

j) Carefully remove and discard the ethanol wash.

Repeat

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

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

m) Remove and discard any remaining ethanol. Remove the tube strip from the 10x Magnetic Separator and immediately add 20 µl Buffer EB.

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

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

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

STOP

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

## 4.7. Post Sample Index PCR Cleanup – SPRIselect – Exome Protocol

| Genome Protocol | Exome Protocol |
|---|---|
| Skip this step and proceed directly to step 4.8 | Proceed with this step 4.7 |

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.

STOP

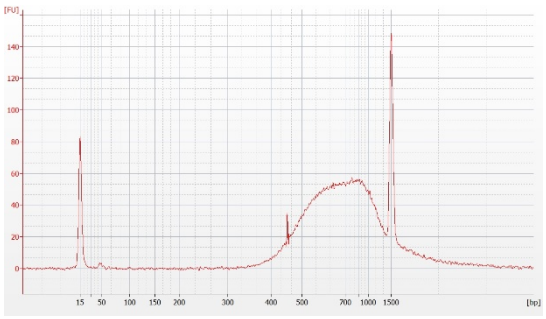
m) Store the tube strip at 4°C for up to 72 h or at –20°C for long-term storage.

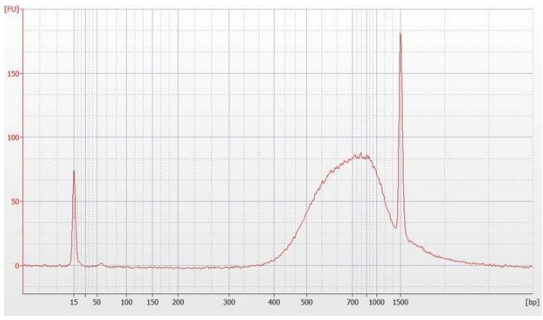
## 4.8. Post Library Construction QC

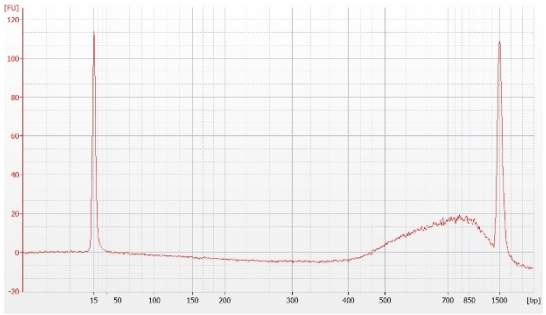
a) **EITHER** Run 1 µl of Genome or Exome Protocol sample on the Agilent Bioanalyzer DNA 1000 chip to determine fragment size. Verify that a library has been generated by looking for a distribution similar to those illustrated below.

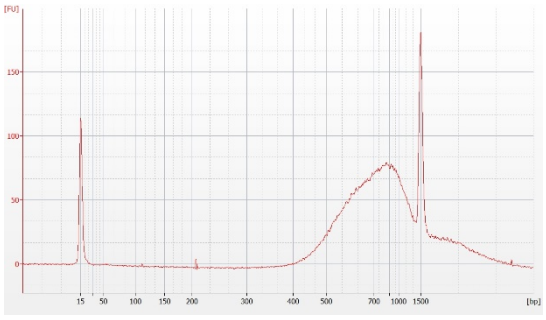
b) For the Exome Protocol, measure the concentration of the library by performing a smear analysis for the region of 100 – 1400 bp. This concentration is then used to calculate the input volume of the library into target enrichment (Protocol Step 5).

Genome Protocol Samples:

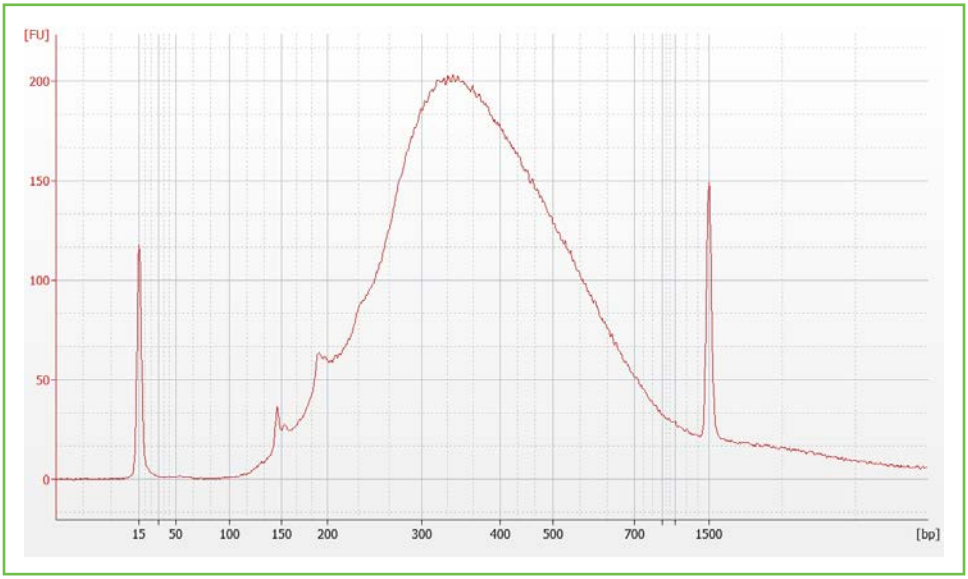


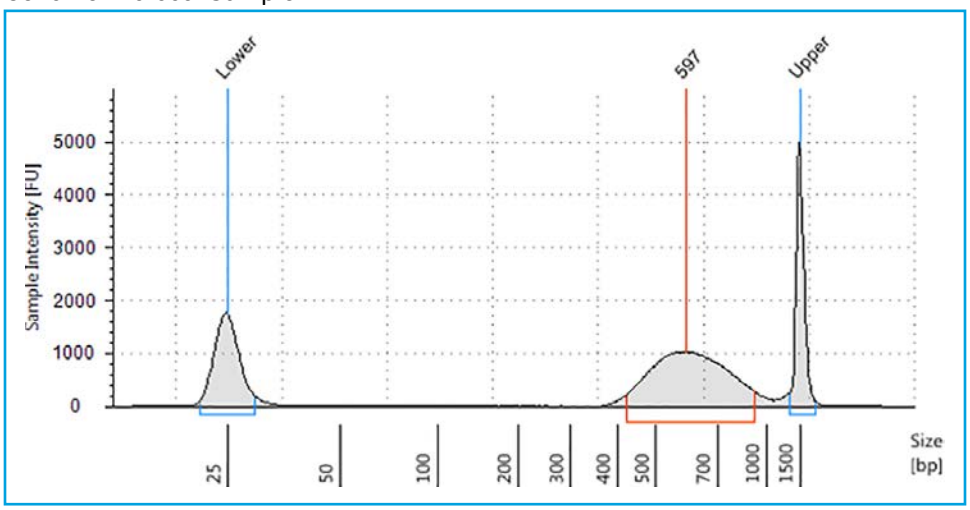



Exome Protocol Sample:

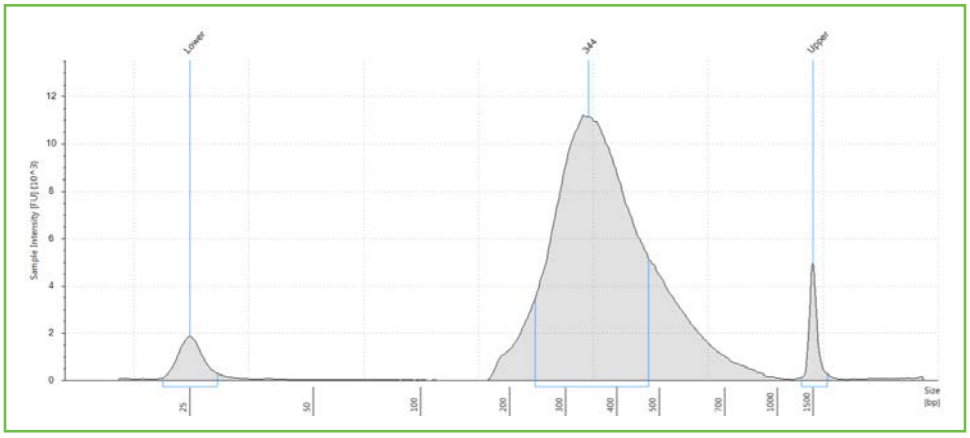


c) **OR** Run 1 µl of Genome or Exome Protocol sample on the Agilent TapeStation D1000 ScreenTape to determine fragment size. Verify that a library has been generated by looking for a distribution similar to those illustrated below.

Genome Protocol Sample:




Exome Protocol Sample:



## 4.9. Post Library Construction Quantification – Genome Protocol

*NOTE*

*Typically a series of 1:40,000, 1:200,000, 1:1,000,000 and 1:5,000,000 of the completed Genome library is required to bring the library within the dynamic range of the assay.*

| Genome Protocol | Exome Protocol |
|---|---|
| Proceed with this step 4.9 | Skip this step and proceed directly to step 5 |

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 | 1X (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 to 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 550 bp.

# Protocol Step 5

# Target Enrichment

Exome Protocol only

Hybridization: Isolate exome-specific fragments for sequencing

5

# 5. Target Enrichment (Hybridization) – Exome Protocol Only

## Tips

Exome target enrichment is performed using a modified version of the Agilent SureSelectXT Target Enrichment System for Illumina Paired-End Sequencing (G7530-90000). Please refer to the manufacturer's website for the most current protocol.

This protocol differs from the Agilent protocol in that each DNA library is lyophilized with blocking oligos before reconstitution and SureSelect ILM Indexing Block 3 is not used. The input amount into target enrichment is higher than the standard Agilent protocol to account for the presence of adaptor dimers. Each DNA library is already indexed with appropriate indices, so addition of the index by PCR after hybridization is not necessary. Samples can also be multiplexed for hybridization.

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

# Getting Started!

## Equilibrate to room temperature before use:

| Item | Storage Location |
|---|---|
| SureSelect Hyb 1 bottle | Follow manufacturer's instructions |
| SureSelect Hyb 2 (Red cap) | Follow manufacturer's instructions |
| SureSelect Hyb 3 (Yellow cap) | Follow manufacturer's instructions |
| SureSelect Hyb 4 (Black cap) | Follow manufacturer's instructions |
| SureSelect Index Block #1 | Follow manufacturer's instructions |
| SureSelect Block #2 (Blue cap) | Follow manufacturer's instructions |

## Place on ice:

| Item | Storage Location |
|---|---|
| SureSelect baits | Follow manufacturer's instructions |
| RNAse block (Purple cap) | Follow manufacturer's instructions |

## Obtain:

| Item | Storage Location |
|---|---|
| SureSelect Capture Reagents | Follow manufacturer's instructions |
| IDT xGen® Universal Blocking Oligo - TS-p5 | Follow manufacturer's instructions |
| IDT xGen® Universal Blocking Oligo - TS-p7(8nt) | Follow manufacturer's instructions |

## Resuspension:

- Universal Blocking Oligos should be resuspended per manufacturer's protocol to 1 µl / reaction

# Target Enrichment (Hybridization) – Exome Protocol Only

| Genome Protocol | Exome Protocol |
|---|---|
| Skip this step 5.1 | Proceed with this step 5.1 |

## 5.1. Library Hybridization

a) In a labeled 8-tube strip, dehydrate 1 µg of prepped library (as determined by Bioanalyzer in step 4.8) with 1 µl TS-p5 blocking oligo and 1 µl TS-p7 (8nt) blocking oligo.

b) Dehydrate the samples at 60°C in a vacuum concentrator.

c) Check samples after 1 h and if samples are not completely dry, continue to monitor every 15 min until completely lyophilized.

d) Reconstitute lyophilized samples with 3.4 µl Nuclease-Free Water.

e) Mix at 1500 rpm for 5 min and then centrifuge briefly.

f) Prepare the Hybridization Solution by adding the reagents shown below at room temperature. Pipette mix thoroughly and centrifuge briefly.

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

g) If precipitate forms, warm the Hybridization Solution at 65°C for 5 min.

h) Prepare the Blocking Mix by adding the reagents shown below at room temperature. Pipette mix thoroughly and centrifuge briefly.

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

i) Add 5.6 µl Blocking Mix to the tube strip containing the reconstituted samples.

j) Pipette mix thoroughly and centrifuge briefly.

k) Place tube strip in thermal cycler and run 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 |

l) Let tubes sit in the thermal cycler for at least 5 min at 65°C. While waiting, finish assembling the capture reagents.

m) Prepare the Capture Baits by adding the reagents shown below at room temperature. Mix and centrifuge briefly.

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

n) Place on ice until the thermal cycling protocol has reached Step 3. Proceed immediately to the next step.

o) Add Hybridization Solution to the tube containing the Capture Baits. Pipette mix thoroughly and centrifuge briefly.

| | **1X (µl)** | **8.8X (µl)** |
|---|---|---|
| **Hybridization Solution** | 13 | 114.4 |

p) Aliquot 20 µl into a new tube strip and centrifuge briefly.

q) Use a multi-channel pipette to quickly and simultaneously transfer 20 µl Capture Baits/Hybridization Solution to tubes at 65°C. Pipette mix 10 times.

***CRITICAL!***

*Tubes should be uncapped for <20 sec.*

r) Cap tube strip with new caps, insert Bio-Rad tube frame and MicroAmp compression pad. Close thermal cycler lid.

s) After 16 h (up to 24 h), proceed to Target Enrichment (Capture).

# Protocol Step 6

# Target Enrichment

Exome Protocol only

Capture: Isolate exome-specific fragments for sequencing

# 6. Target Enrichment (Capture) – Exome Protocol Only

## Tips

### General

Capture of the hybridized exome libraries is performed using a modified version of the Agilent SureSelectXT Target Enrichment System for Illumina Paired-End Sequencing (G7530-90000). Please refer to the manufacturer's website for the most current protocol.

The steps in this section differ from the Agilent protocol in that each DNA library is already indexed with appropriate indices, so addition of the indexing tag by PCR after hybridization is not necessary.

# Getting Started!

## Equilibrate to room temperature before use:

| Item | Storage Location |
|---|---|
| Post Capture PCR Primers | –20°C |
| Beckman Coulter SPRIselect Reagent | Follow manufacturer's instructions |
| Agilent Bioanalyzer High Sensitivity Kit | Follow manufacturer's instructions |
| Agilent TapeStation High Sensitivity D1000 ScreenTape and Reagents (if used for QC) | Follow manufacturer's instructions |

## Place on ice:

| Item | Storage Location |
|---|---|
| Post Capture PCR Master Mix | –20°C |
| Kapa DNA Quantification Kit for Illumina Platforms | Follow manufacturer's instructions |

## Obtain:

| Item | Storage Location |
|---|---|
| SureSelect Capture Reagents | Follow manufacturer's instructions |
| DynaBeads® MyOne™ Streptavidin T1 beads | Follow manufacturer's instructions |
| Qiagen Buffer EB | Follow manufacturer's instructions |

## Warm to 65°C:

- Thermal cycler or heat block for tube strips

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

# Target Enrichment (Capture) – Exome Protocol Only

| Genome Protocol | Exome Protocol |
|---|---|
| Skip steps 6.1 – 6.6 | Proceed with this step 6.1 |

## 6.1. Preparing Streptavidin Beads

a) Place 210 µl SureSelect Wash Buffer 2 into each of 3 8-tube strips.

b) Place tube strips in thermal cycler at 65°C with lid at 80°C until use in Step 6.2.

c) Vigorously resuspend Dynabead MyOne Streptavidin T1 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. Add 210 µl Dynabead MyOne Streptavidin T1 beads to 1.6 ml eppendorf tube.

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 beads into labeled tube strip wells.

## 6.2. Capturing Hybridized DNA & Washing

a) Maintain the tube strip at 65°C while using a multi-channel 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 the wells.

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

c) Centrifuge the tube strip briefly.

d) Place tube strip into a 10x™ Magnetic Separator in the High position 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 tube strip on a 96-well plate mixer, mixing vigorously (1500 rpm) for 15 min at room temperature.

h) Centrifuge the tube strip briefly.

i) Place tube strip into a 10x™ Magnetic Separator in the High position until the solution is clear.

j) Carefully remove and discard the supernatant.

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

l) Incubate the tube strip on a 96-well plate mixer, mixing vigorously (1500 rpm) for 10 min at 65°C.

m) Centrifuge the tube strip briefly.

n) Place tube strip into a 10x Magnetic Separator in the High position 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) Remove and discard any remaining wash buffer. Remove the tube strip from the 10x Magnetic Separator and immediately add 22.5 µl Nuclease-Free Water to each sample well. Pipette mix until beads are fully resuspended.

r) Proceed immediately to 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™ Post Capture PCR Mix and Primers.*

| 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** |

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

c) Run the Post Capture PCR protocol. Run time is ~20 min.

| Lid Temperature | Reaction Volume | |
|---|---|---|
| 105°C | 50 µl | |
| | | |
| **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 | 2:00 |
| **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.

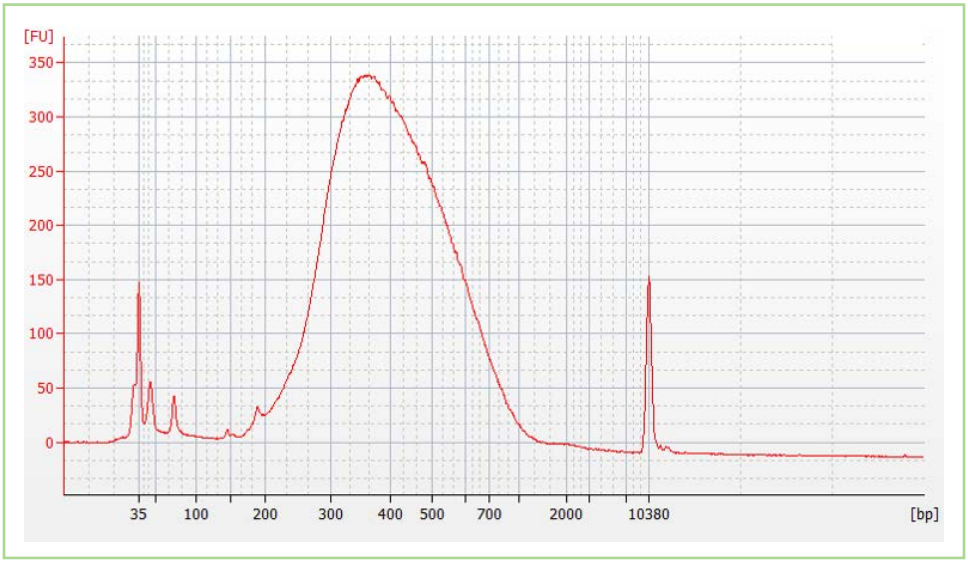
l) Transfer 20 µl of sample to a new tube strip.

STOP

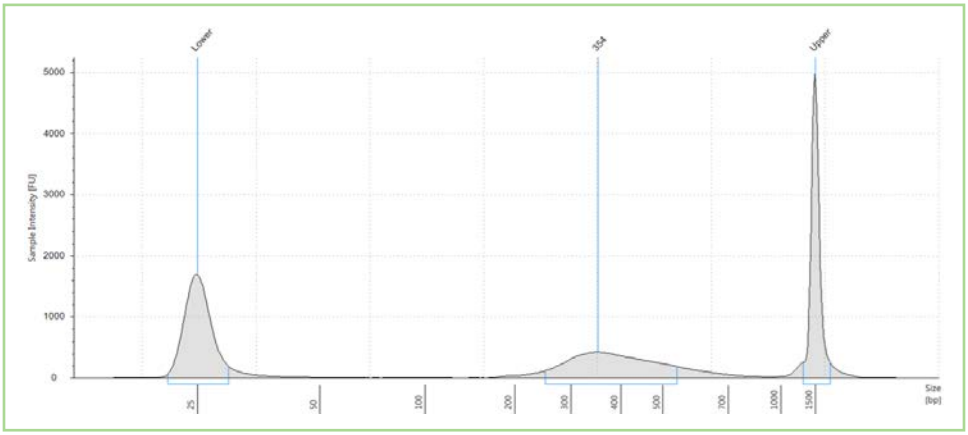
m) Store the tube strip at 4°C for up to 72 h or at –20°C for long-term storage.

## 6.5. Post Target Enrichment Library QC

a) **EITHER** Run 1 µl of Exome Protocol 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 2 µl of Exome Protocol sample on the Agilent TapeStation High Sensitivity D1000 ScreenTape to determine fragment size.



## 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** | **1X (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 to 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.

# 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 Genome and Exome libraries to deliver the full value of the Chromium Genome Solution.

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

| 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) | 90 Million (45 Million read pairs) |
| Targeted Deduped Depth | >30x | >60x |

## 7.2. Sequencing Run Parameters

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

b) Genome and 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 Genome Protocol | Recommended Number of Cycles for Exome Protocol |
|---|---|---|
| Read 1 | 150 cycles | 100 cycles |
| i7 index | 8 cycles | 8 cycles |
| i5 index | 0 cycles | 0 cycles |
| Read 2 | 150 cycles | 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, but the 10x Sample Index name (Genome Index plate well ID) is needed for the bioinformation if running more than one sample.

# Practical Tips & Troubleshooting

Processing Fewer than 8 Reactions

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

Pipetting Gel Beads & GEMs

Surrogate Fluid

Reagent Clogs during GEM Generation

Chromium™ Controller Errors

Glossary of Terms

8

# 8. Practical Tips & Troubleshooting

## 8.1. Processing Fewer than 8 Reactions

a) Puncture foil seals in the Gel Bead Strip as needed for a run.

b) Store any unused Gel Beads at –80°C and avoid more than 4 freeze-thaw cycles.

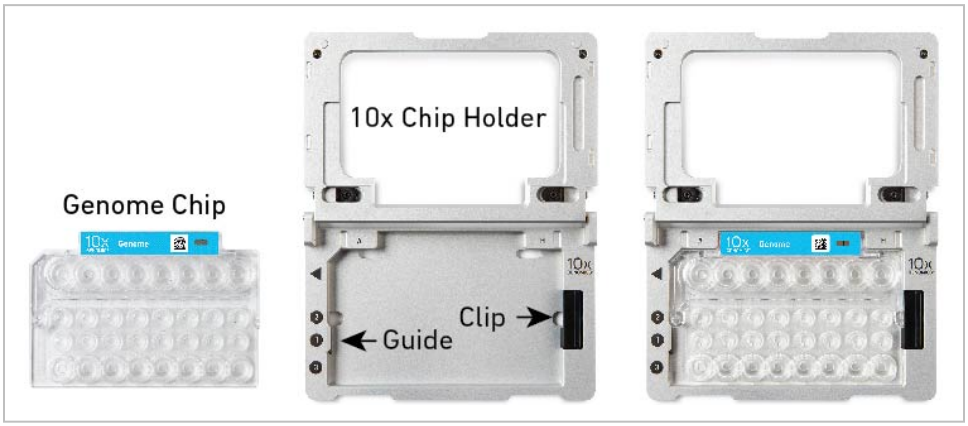
c) Never store Gel Beads at –20°C.

d) Reagent volumes should be calculated with a 10% excess of 1X values quoted in the protocol. e.g. For 3 samples, multiply the 1X volume quoted in the protocol by 3.3 to determine the suitable volume to prepare. Using larger reagent excesses may reduce the total number of reactions that can be run using one kit.

## 8.2. Assembling a Chip, 10x™ Chip Holder & 10x™ Gasket

a) Align the notch on the upper left corner of the Chromium™ Chip with the notch on the 10x Chip Holder and insert the left-hand side of the Chromium Chip under the guide.

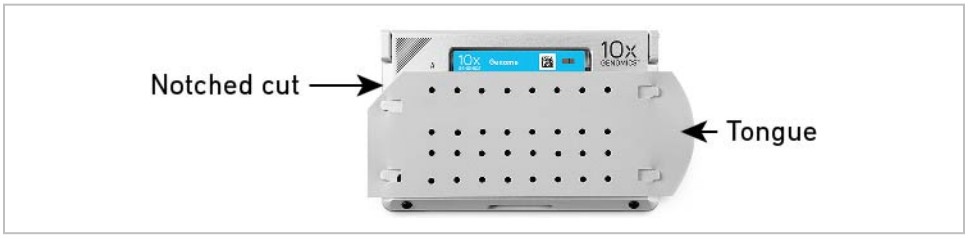
b) Depress the right-hand side of the Chromium Chip until the spring-loaded clip engages the Chromium Chip.




c) Close the hinged lid of the 10x Chip Holder. After loading the Chromium Chip, the 10x Chip Holder should lay flat on the bench top with the lid closed.

d) Position the assembly so that the Partitioning Oil wells (row labeled 3) are toward you and identify the rows labeled 1, 2 and 3 for correct addition of the reagents.

e) After the reagents have been added, attach a 10x™ Gasket by holding the tongue (curved end, to the right) and with the notched cut at the top left corner, hook it on the left-hand tabs of the 10x Chip Holder. Gently pull the 10x Gasket toward the right and hook it on the two right-hand tabs. Avoid touching the smooth side of the 10x Gasket and do not press down on the top of the 10x Gasket after attachment.




Click back to Loading the Genome Chip

## 8.3. Pipetting Gel Beads & GEMs

a) After vortexing, remove the Gel Bead Strip from the 10x™ Vortex Adapter and flick the Gel Bead Strip in a sharp, downward motion to ensure maximum Genome Gel Bead recovery. Confirm that there are no bubbles at the bottom of the tube.

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

c) Best practices for recovering adequate volume of Gel Beads from the Gel Bead Strip include the following:

   i. Set a pipette to the volume being pipetted and, without engaging the plunger, puncture the foil seal on the Gel Bead Strip. The pipette tips should extend no more than 2 mm below the seal.

   ii. Once the holes are formed, raise the pipette tips above the seal and engage the plunger.

   iii. Lower the tips to the bottom of the wells and widen the opening by gently rocking the tips back and forth, keeping the plunger engaged. Widening the foil seal opening allows the pipette tips to reach the bottom of the Gel Bead Strip wells. This is important for recovering the full volume of Gel Beads required for optimal performance.

   iv. With the pipette tips still in the Gel Bead Strip, very slowly aspirate the required volume of Gel Beads. After aspiration stops, leave the pipette tips in the wells for an additional 5 sec to allow pressure to equilibrate.


d) If the required volume of beads cannot be recovered, place the pipette tips against the sidewalls of the Gel Bead Strip wells and slowly dispense the Gel Beads back into the strip. Take care not to introduce bubbles into the wells and verify that the pipette tips contain no leftover Gel Beads. Attempt to withdraw the full volume of beads again by pipetting slowly.

e) After the completion of a Chromium™ Controller run, the hinged lid of the 10x™ Chip Holder is folded back to expose the wells at a 45-degree angle. The GEMs should be aspirated from the lowest points of the Recovery Wells (row labeled ◀) without creating a seal between the tips and the bottom of the wells. Avoid introducing air bubbles.


Click back to Loading the Genome Chip

## 8.4. Surrogate Fluid

Surrogate Fluid is glycerol in a ~50% volume/volume aqueous solution. It is critical all unused wells in Rows labeled 1, 2 and 3 of the Genome Chip contain Surrogate Fluid prior to running the Chromium Controller. If the amount of Surrogate Fluid provided in the Library Kit is insufficient, the following commercially available glycerol solution can be used as a substitute:

Ricca Chemical Company, Glycerin (glycerol), 50% (v/v) Aqueous Solution, PN-3290-32

Alternatively, additional Surrogate Fluid can be made from a stock solution of glycerol as follows:

a) Mix an equal volume of water and 99% Glycerol, Molecular Biology Grade.
b) Filter through a 0.2 µm filter.
c) Store at –20°C in 1 ml LoBind tubes.
d) Surrogate Fluid should be equilibrated to room temperature before use.

## 8.5. Reagent Clogs during GEM Generation

GEM reagents are manufactured in a cleanroom environment to minimize the level of fibers and debris in GEM reagents that could cause a clog during GEM generation.

It is also important for users to minimize exposure of reagents, chips, and gaskets to sources of fibers such as reagent reservoirs, KimWipes, repeat-usage of flip-cap tubes, and the general laboratory environment. When care is taken to minimize the introduction of additional debris, the clog rate is typically very low. In the unlikely event that a clog occurs during GEM generation, **it is recommended that the sample be remade.**

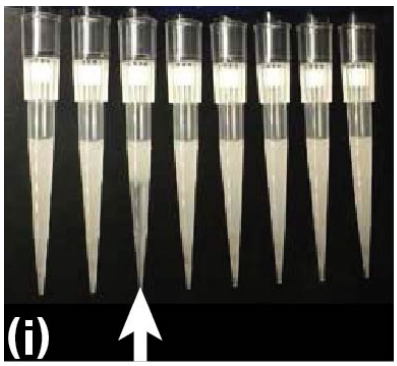
There are several ways to identify if a clog has occurred. If any of the following occur, take a picture and send it to support@10xgenomics.com for further assistance. Continue processing the remaining samples:

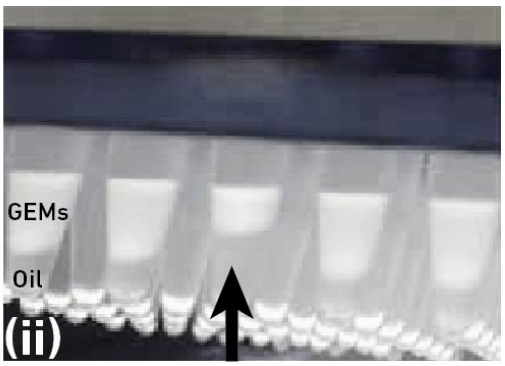
a) After removing a Chromium™ Chip from the Chromium Controller, one or more wells contain an abnormally high volume.

b) During GEM recovery:

   i. When transferring GEMs from the Chromium Chip to a PCR plate, there is excess Partitioning Oil in a pipette tip after aspiration. Note the excess Partitioning Oil present in the third tip from the left (below left, arrow).

   ii. Excess Partitioning Oil can also be observed by comparing the volume uniformity in the PCR plate. After the GEMs are transferred to a PCR plate, a clog occurred if the Partitioning Oil volume (clear, bottom of well) in one or more wells is increased compared to other wells (below right, arrow).

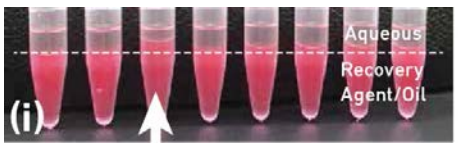


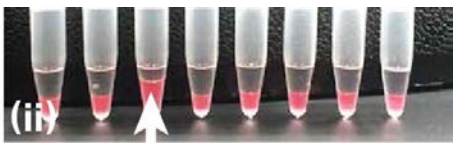




c) After transfer of the GEMs + Recovery Agent to a tube strip:

   i. Wells with decreased aqueous sample indicate a clog during GEM generation when compared to wells with other normal samples (below left, arrow).

   ii. After aspirating the designated volume of Recovery Agent/Partitioning Oil, a greater volume of Partitioning Oil (pink) remaining in the PCR tubes (below right, arrow) also indicates a clog occurred.



## 8.6. Chromium™ Controller Errors

If the Chromium Controller fails to start, an error tone will sound and one of the following error messages will be displayed:

a) **Chip not read – Try again**: Eject the tray, remove and/or reposition the 10x™ Chip Holder assembly and try again. If the error message is still received after trying this more than twice, contact support@10xgenomics.com for further assistance.

b) **Check Gasket**: Eject the tray by pressing the eject button to check there is a 10x Gasket on the Chromium Chip. In the case the 10x Gasket installation was forgotten, install and try again. In the case a 10x Gasket was already installed, remove, reapply, and try again. If the error message is still received after trying either of these more than twice, contact support@10xgenomics.com for further assistance.

c) **Pressure not at Setpoint:**

   i. If this message is received within a few seconds of starting a run, eject the tray by pressing the eject button and check for dirt or deposits on the 10x Gasket. If dirt is observed, replace with a new 10x Gasket and try again. If the error message is still received after trying this more than twice, contact support@10xgenomics.com for further assistance.

   ii. If this message is received after a few minutes into the run, it is likely one or more of the reagents was not loaded into the Chromium Chip. In this case, the Chromium Chip must be discarded. **Do not try running this Chromium Chip again as this will damage the Chromium Controller**.

d) **CAUTION: Chip Holder not Present**: Eject the tray by pressing the eject button to check there is a 10x Chip Holder encasing the Chromium Chip. In the case the 10x Chip Holder was forgotten, install with a 10x Gasket in place, and try again. If the error message is still received after a 10x Chip Holder is confirmed as in place, contact support@10xgenomics.com for further assistance.

e) **Invalid Chip CRC Value**: This indicates the Chromium Chip has encountered an error, should not be run, and must be discarded. Contact support@10xgenomics.com for further assistance.

Click back to Running the Chromium Controller

## 8.7. Glossary of Terms

### 10x™ Barcode

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

### Chromium™ Genome Chip

The Chromium Genome Chip is a microfluidic chip specifically designed to run the Genome Protocol in the Chromium Controller. The Genome Chip is indicated by a blue label at the top of the chip. Other chips used with the Chromium System include the Chromium Single Cell 3' Chip.

### Gel Beads

Gel Beads are the foundation of 10x Genomics®' technology, and are beads functionalized with millions of copies of a 10x Barcoded primer. Gel Beads are provided in 8-reaction Gel Bead Strips.

### GEM

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

### GemCode™ Technology

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

### High Molecular Weight Genomic DNA

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

### Linked-Reads

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

### Long-range Information

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