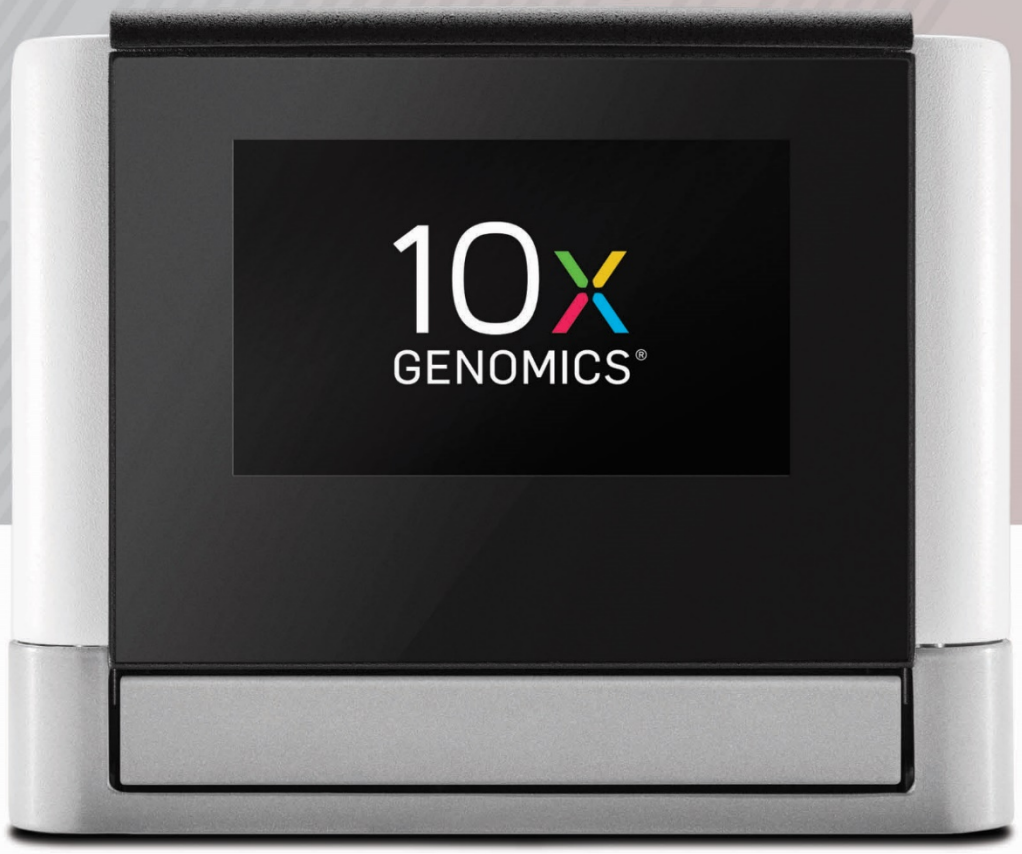
10x Genomics®

Sample Preparation Demonstrated Protocol

DNA Extraction from Fresh Frozen Tissue



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Notices

Manual Part Number

CG00016 Rev B

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Customer Information and Feedback

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

Email: techsupport@10xgenomics.com

10x Genomics

7068 Koll Center Parkway

Suite 401

Pleasanton, CA 94566 USA

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HMW gDNA Extraction

From fresh frozen tissue

1. Overview & Getting Started

High Input Genomic DNA Length Results in Optimal Performance of the Chromium™ Genome Protocols

The Chromium Genome Protocols generate long-range information across the length of individual DNA molecules. Starting the process with High Molecular Weight (HMW) genomic DNA (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 >50 kb.

This Demonstrated Protocol outlines the recommended method for HMW gDNA extraction from ~25 mg tissue. No additional reagents or consumables are required other than those listed in the Chromium Genome User Guides.

This Demonstrated Protocol can produce gDNA with an average size of ~20-80 kb when analyzed on a pulsed-field gel, and typically ~40 kb after the Chromium Genome Protocols. There are many variables that will affect gDNA quality in these sample types, including sample age, transportation methods, additives, freezing method, etc.

Getting Started!

This Demonstrated Protocol utilizes the Qiagen MagAttract HMW Kit (catalog no. 67653), with minor modifications. Please refer to the manufacturer's brochure (Qiagen MagAttract HMW DNA Kit Handbook 08/2013) for reagent preparation, storage, and troubleshooting.

Preparation:

- Set a thermomixer to 56°C

Required Reagents:

- Qiagen MagAttract HMW Kit
  - Buffer ATL – if precipitate is visible, warm at 37°C and mix to dissolve
  - Buffer AL
  - MagAttract Suspension G
  - Buffer MB
  - Buffer MW1
  - Buffer PE
  - Buffer AE
  - Proteinase K
  - RNase A
  - Nuclease-free water

Required Plastics:

- Eppendorf DNA LoBind Tube Microcentrifuge Tube, 1.5 ml (PN-022431021)
- Eppendorf DNA LoBind Tube Microcentrifuge Tube, 2.0 ml (PN-022431048)

Required Plastics:

- Eppendorf ThermoMixer® C (PN-5382000015)
- Eppendorf SmartBlock™ 2.0 ml, thermoblock for 24 Reaction Vessels (PN-5362000035)
- Thermo Fisher DynaMag™-2 magnet (PN-12321D)

2. HMW gDNA Extraction Protocol

NOTE *This Protocol was demonstrated using the same commercially sourced fresh-frozen breast tumor sample for each replicate.*

2.1. Lysing the Fresh Frozen Tissue

a) Excise ~25 mg tissue and place in a 2 ml sample tube.

NOTE *Weighing the tissue is the most accurate way to determine the amount.*

b) Add 200 µl Buffer ATL to the sample.

c) Add 20 µl Proteinase K and mix by pulse-vortexing 3 times at the highest speed setting.

d) Incubate the sample at 56°C and 900 rpm overnight (12-16 h) until the tissue is completely lysed

2.2. Extracting HMW gDNA

a) Centrifuge samples briefly to remove condensation that may have formed on the lid of the tube and to ensure any incompletely lysed tissue particles are settled at the bottom of the tube.

NOTE *If pieces of insoluble material are still present, centrifuge at the speed of 20,000 x g for 2 min and transfer the supernatant into a clean 2 ml sample tube using a wide-bore tip.*

b) Transfer 200 µl of the lysate to a new 2 ml sample tube.

c) Add 4 µl RNase A and mix by pulse-vortexing 3 times at the highest speed setting. Incubate for 2 min at room temperature.

d) Add 150 µl Buffer AL to the sample. Mix by inversion 5 times and centrifuge briefly.

e) Add 280 µl Buffer MB to the sample.

f) Vortex the MagAttract® Suspension G for 60 sec and add 40 µl to the sample.

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

g) Incubate at 15-25°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 a magnetic rack. Add 700 µl Buffer MW1 directly to the bead pellet. Incubate at 15-25°C and 1400 rpm for 1 min.

Repeat j) Repeat steps h and i for a total of 2 Buffer MW1 washes.

k) Centrifuge the tube briefly and place on a 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 15-25°C and 1400 rpm for 1 min.

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

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

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

CRITICAL!

The timing of the next step is extremely important. If a multichannel pipette is not available, ensure that each tube has the exact same incubation time. Do not exceed 60 sec.

o) Carefully add 700 µl nuclease-free water down the side of the tube **opposite** the magnetic pellet. Incubate for **exactly** 60 sec. Promptly remove and discard the supernatant.

Repeat

p) Repeat step n and o for a total of 2 nuclease-free water washes.

q) Remove the sample tubes from a magnetic rack. Add 150 µl Buffer AE directly to the bead pellet and incubate at 15-25°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 HMW gDNA to a new 1.5 ml low-bind screw-cap tube.

STOP

t) Store the extracted HMW gDNA sample at 4°C for up to 2 weeks or at –20°C for up to 6 months.

3. Results

NOTE

The table below outlines yields from ~25 mg tissue samples.

Replicate	Tissue (mg)	ng/µl	Yield (µg)
1	19.4	101	15.2
2	33.8	214	32.1
3	24.2	104	15.6
4	18.7	76.7	11.5

NOTE

Pulsed-field gel results (below) from 4 replicates of this Demonstrated Protocol show the majority of gDNA is ~40-50 kb. Demonstrated Protocols for running pulsed-field gels and other DNA quality control (QC) methods are available from 10x Genomics®.

A 40 kb high-pass size selection using the Sage Blue Pippin or PippinHT is recommended if available, as the Chromium™ Genome Protocol performance will increase. The 10x Genomics Size Selection Demonstrated Protocol for removal of DNA <20 kb or DNA <40 kb from given samples should be consulted for further information.