10x Genomics®

Sample Preparation Demonstrated Protocol

DNA Extraction from Whole Blood

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Notices

Manual Part Number

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Table of Contents

Demonstrated Protocol

HMW gDNA Extraction

From whole blood

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 200 µl fresh whole blood. No additional reagents or consumables are required other than those listed in the Chromium Genome User Guides.

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.

Required Reagents:

- Qiagen MagAttract HMW Kit
  - 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 fresh blood collected in an EDTA purple cap tube and delivered overnight on ice to the laboratory. The blood was then stored for 2 days at 4°C prior to processing. Successful results have also been demonstrated using whole blood collected in an EDTA purple cap tube and stored for >1 month at either –20°C or –80°C.

2.1. Lysing Whole Blood

a) Add 20 µl Proteinase K to the bottom of a 2 ml sample tube.

b) Add 200 µl whole blood to the sample tube.

c) Add 4 µl RNase A and 150 µl Buffer AL to the sample tube. Mix by pulse-vortexing 3 times at the highest speed setting.

d) Incubate the sample for 30 min at 25°C.

2.2. Extracting HMW gDNA

a) Centrifuge samples briefly to remove condensation that may have formed on the lid of the tube.

b) Vortex the MagAttract® Suspension G for 60 sec 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.

c) Add 280 µl Buffer MB. Incubate at 15-25°C and 1400 rpm for 3 min.

d) 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.

e) 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

f) Repeat steps d and e for a total of 2 Buffer MW1 washes.

g) 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.

h) 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

i) Repeat steps g and h for a total of 2 Buffer PE washes.

j) 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.

CRITICAL!

Leave the sample tubes on a 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 multichannel pipette is not available, ensure that each tube has the exact same incubation time. Do not exceed 60 sec.

k) 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

l) Repeat step j and k for a total of 2 nuclease-free water washes.

m) 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.

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

o) 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.

p) 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

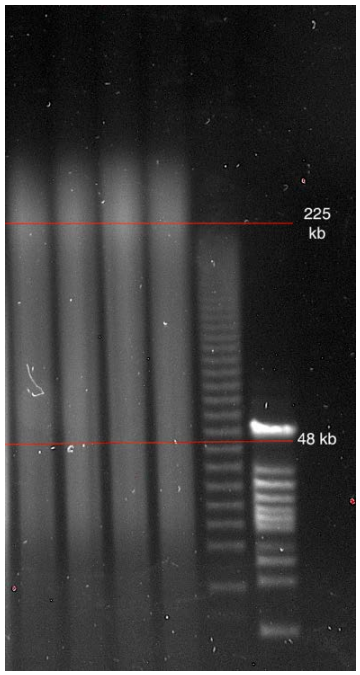
NOTE

Starting from 200 µl fresh whole blood, the expected yield is ~4 µg HMW gDNA.

Replicate	Concentration (ng/µl)	Total Yield (µg)
1	25.9	3.9
2	26.6	3.9
3	26.9	4.0
4	25.7	3.9

NOTE

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



4. Troubleshooting

a) If suboptimal yield or gDNA quality is observed, the quality of the starting sample may be compromised. This is routinely observed with blood samples that are coagulated or not collected and stored in a suitable tube. Ideally, a blood sample should be re-drawn into an EDTA tube.

b) If the isolated gDNA is <50 kb and another sample is not available, the Sage Science Blue Pippin or PippinHT instruments are recommended for size selection. 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.