10x Genomics®

Sample Preparation Demonstrated Protocol

DNA Extraction from Fresh Frozen Tissue

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Notices

Manual Part Number

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From fresh frozen tissue

1. Overview

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 a method for HMW gDNA extraction from fresh frozen tissue. This Protocol can produce gDNA with an average size of >200 kb when analyzed on a pulsed-field gel, and typically >80 kb after the Chromium Genome Protocols. There are many variables that will affect gDNA quality in sample types, including sample age, transportation methods, type of tissue, additives, freezing method, etc.

This Protocol outlines HMW gDNA extraction from fresh frozen breast tumor tissue. While results are expected to be similar across many tissue types, this Protocol has not yet been extensively tested. Importantly, there are tissue types for which HMW gDNA extraction is universally difficult (e.g. liver, due to high enzyme activity) and also yield sub-optimal results with this Protocol.

2. Getting Started!

2.1. Tips & Safety

To ensure accurate and consistent tissue sample mass measurements, pre-weigh each tube individually before thawing the tissue. Cap the empty tube and weigh upside down. Place the thawed tissue sample on the cap of the tube and again weigh upside down. (Placing the sample in different parts of the tube will result in inconsistent measurements).

CRITICAL!

Follow tissue and material supplier recommendations and local laboratory procedures and regulations for the safe handling, storage and disposal of biological materials.

2.2. Materials

Supplier	Description	Part Number (US)
Sigma	Nuclei PURE Prep Kit Lysis Buffer 10% Triton X-100 Ethylenediaminetetraacetic acid disodium salt (EDTA) Dithiothreitol (DTT) N-Laurylsarcosine sodium salt Ethanol, Pure (200 Proof, anhydrous)	 NUC-201 E5134 D9779 L9150 459836-500ML
Bio-Rad	10% Tween 20	1610781
Corning	Phosphate-Buffered Saline (PBS) 1X	21-040-CV
Qiagen	Proteinase K Buffer AE	19131 19077
Teknova	1M Tris-HCl, pH 8.3	T1083
Beckman Coulter	SPRIselect Reagent Kit	B23317
Eppendorf	DNA LoBind Tubes, 2.0 ml ThermoMixer® C SmartBlock™ 2.0 ml, thermoblock for 24 Reaction Vessels	022431048 5382000015 5362000035
Thermo Fisher Sci	Kimble™ Kontes™ Pellet Pestle™ 1.5 ml tubes DynaMag™-2 Magnet Argos Technologies RotoFlex™ Tube Rotator Nuclease-Free Water Qubit® dsDNA BR Assay Kit Qubit® Assay tubes Qubit 3.0 Fluorometer	K749520-0090 12321D 22-505-001 AM9937 Q32853 Q32856 Q33216
-	Refrigerated Benchtop Centrifuge	-

2.3. Preparation – Buffers

a) Freshly prepare (or freshly thaw aliquot) a 1 M aqueous solution of Dithiothreitol (DTT). Freshly prepare Nuclei Isolation Buffer by adding reagents in the order shown below. Mix thoroughly and place on ice prior to starting the Protocol. 500 µl Nuclei Isolation Buffer is needed for each sample extraction.

Nuclei Isolation Buffer	**Example volumes sufficient for 4 rxns + excess**
Sigma Nuclei PURE Prep Kit: Lysis Buffer	2.2 ml
1 mM DTT	2.2 µl
Sigma Nuclei PURE Prep Kit: 10% Triton X-100	22 µl

b) Prepare Digestion Buffer by adding reagents in the order shown below. Stock 0.5 M EDTA, pH 11 should be prepared ahead of time. Digestion Buffer can be made in bulk and stored at room temperature. Place an aliquot of sufficient Digestion Buffer on ice prior to starting the Protocol. 70 µl Digestion Buffer is needed for each sample extraction.

Digestion Buffer	**Volume / Mass**	**Final Concentration**
0.5 M EDTA, pH 11	2 ml	20 mM
Teknova 1M Tris-HCl, pH 8.3	100 µl	2 mM
Sigma N-Laurylsarcosine sodium salt	149 mg	10 mM
Nuclease-Free Water	Fill to 50 ml	-
Total	**50 ml**	-

c) Prepare Sample Elution Buffer by adding reagents in the order shown below. Sample Elution Buffer can be made in bulk and stored at room temperature. 50 µl Sample Elution Buffer is needed for each sample extraction.

Sample Elution Buffer	**Volume (µl)**
Qiagen AE Buffer	990
Bio-Rad 10% Tween 20	10
Total	**1000**

d) Place an aliquot of sufficient Phosphate-Buffered Saline (PBS) on ice prior to starting the Protocol. 70 µl PBS is needed for each sample extraction.

e) Prepare 70% Ethanol (10 ml for 4 samples).

3. HMW gDNA Extraction Protocol

3.1. Fresh Frozen Tissue Preparation

a) Thaw the fresh frozen tissue by placing the tube on ice (typically 2-3 min).

b) Cut a piece of tissue approximately 0.5 cm^3 (this is typically approximately 25 mg).

NOTE

The amount of nuclei in each sample will differ greatly depending on the tissue types, etc. The minimum and maximum number of nuclei required for optimal application performance are not yet determined.

c) Place each piece of tissue in a separate 1.5 ml Kimble™ Kontes™ Pellet Pestle™ tube and maintain on ice.

3.2. Nuclei Isolation

a) Centrifuge samples briefly to move the tissue to the bottom of the tube.

b) Add **500 µl** cold Nuclei Isolation Buffer to each tube.

c) Homogenize the tissue by gently moving the pestle straight up and down **10 times.** Do not twist the pestle.

d) Centrifuge the tube briefly.

e) Place the tube on ice for **1 min** to allow the tissue to settle to the bottom of the tube.

f) Using a **wide-bore** pipette tip, transfer as much of the supernatant as possible into a new 2.0 ml tube. Leave as much as the tissue behind as possible. A small amount of tissue carry-over is acceptable, as this will be removed during the DNA purification step.

Optional

g) Save 10 µl supernatant in a tube on ice to quantify or visually inspect the nuclei (using Trypan Blue staining, for example).

h) Centrifuge in a chilled centrifuge (**4°C**) at **500 x g** for **5 min**.

3.3. Nuclei Digestion

a) Remove and discard the supernatant from the tube without disturbing the nuclei pellet.

b) Add **70 µl** cold PBS and tap the tube lightly to dislodge the nuclei pellet.

NOTE

"Tap to mix" in the following two steps is to gently tap the side of the tube 1-2 times with a finger. Thorough mixing is not necessary as the digestion will take place on a rotator, which will ensure adequate sample mixing.

c) Add **10 µl** Proteinase K, tap to mix.

d) Add **70 µl** cold Digestion Buffer, tap to mix.

e) Rotate sample on a RotoFlex™ Tube Rotator using **speed setting 10** for **2 h** at **room temperature**. Ensure the samples are positioned to rotate end-over-end.

f) Centrifuge the tube briefly. The sample should be viscous.

3.4. DNA Purification

a) Measure the actual volume of the sample with a **wide-bore** pipette tip.

b) Add Tween 20 to the sample to a final concentration of 0.1% (e.g. add 1.5 µl 10% Tween 20 to 150 µl sample, measured in step a) and pipette mix **5 times** using a **wide-bore** pipette tip.

c) Vortex the SPRIselect Reagent until fully resuspended. Add an equal volume of 1X SPRIselect Reagent to the sample volume (e.g. for the example in step b, add 152 µl SPRIselect Reagent) and rotate on a RotoFlex™ Tube Rotator using **speed setting 10** for **20 min**. Ensure the samples are positioned to rotate end-over-end.

d) Centrifuge the tube briefly and place on a DynaMag™-2 Magnetic Rack for ~**1 min** to allow bead capture**.**

e) Carefully remove and discard the supernatant. Take care not to disturb the bead pellet.

f) While the tube is on the magnetic rack, fill the tube with 70% Ethanol 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 briefly and place on the magnetic rack for ~**1 min** to allow bead capture. Remove and discard residual supernatant. Do not allow the bead pellet to dry.

j) Remove the sample from the magnetic rack. Add **50 µl** Sample Elution Buffer directly to the bead pellet and incubate at **room temperature** for **5 min**.

k) Further incubate the tube at **25°C** and **1400 rpm** for **3 min**.

l) Centrifuge the tube briefly and place on the magnetic rack for ~**1 min** to allow bead capture.

m) Using a **wide-bore** pipette tip, transfer the purified gDNA sample to a new 2.0 ml tube.

NOTE

It is optional to repeat steps j-m to recover additional gDNA. 10x Genomics® typically elutes twice. The eluents are typically not combined, but the second eluent is retained as additional sample, if needed.

Optional

n) Quantify the sample using the Qubit® dsDNA BR Kit, taking multiple measurements from different parts of the tube. This Protocol generates a gDNA solution that is not viscous and that is uniformly distributed throughout the tube. Different tissue or sample types may produce different results.

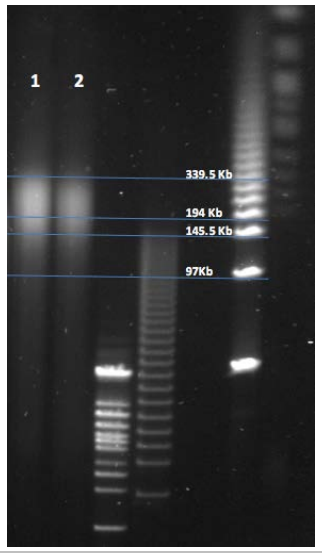
o) Store the purified gDNA sample at **4°C** for up to **2 weeks** or at **–20°C** for up to **6 months**.

4. Results

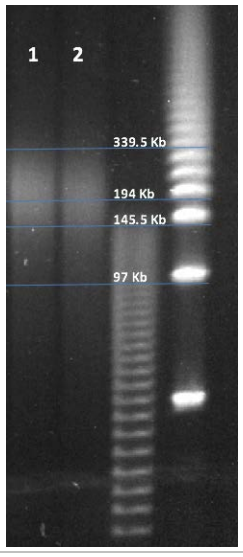
This Protocol is demonstrated using fresh frozen breast tumor tissue. Two samples were processed and two elutions were performed on each sample. All samples were analyzed on a pulsed-field gel. A subset of the resulting gDNA was processed with Chromium™ Genome Reagent Kits (v1 Chemistry) and sequenced on an Illumina HiSeq 4000. The mean DNA size for each sample was >80 kb, which is the highest level of sample quality as defined in the 10x Genomics® Technical Note: Sample Preparation Recommendations for the Chromium™ Genome Kit (CG00045).

Replicate	Starting Tissue Mass	Total DNA Mass	DNA Size (PFGE)	DNA Size (Chromium Genome)	DNA Quality Level
1	28.7 mg	1625 ng	>150 kb	137 kb	5
2	28.7 mg	1443 ng	>150 kb	115 kb	5

Elution 1 of Replicates 1 and 2



Elution 2 of Replicates 1 and 2



Sequencing Results of Replicates 1 and 2