10x Genomics®

Sample Preparation Demonstrated Protocol

Salting Out Method for DNA Extraction from Cells

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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 gDNA extraction from suspension cells. 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 sample, additives, freezing method, etc.

This Protocol outlines gDNA extraction from suspension cells and although not demonstrated here, results are expected to be similar with adherent cells.

2. Getting Started

2.1. Tips & Safety

Best practices for handling any cell line includes using sterile techniques, nuclease-free reagents and consumables, minimizing pipetting steps, and using wide-bore pipette tips, when possible, to minimize cell damage prior to lysis.

Best practices for handling HMW gDNA include:

- 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 when transferring HMW gDNA for accuracy.

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

2.1. Literature

a) This Protocol is an adaptation of Miller, S.A., Dykes, D.D., Polesky, H.F. "A Simple Salting Out Procedure for Extracting DNA from Human Nucleated Cells" *Nucleic Acids Research* **1988**, *16*, 1215.

2.2. General Materials

Supplier	Description	Part Number (US)
Teknova	1M Tris-HCl, pH 8.0	T5088
Qiagen	Proteinase K, 20 mg/ml	19133
Sigma-Aldrich	Sodium Chloride Solution, 5M Ethanol, Pure	S6546 459836
Thermo Fisher Sci	0.5M EDTA pH 8.0 10% SDS TE Buffer Polypropylene centrifuge tubes, 15 ml	AM9260G 15553-027 12090-015 05-539-12
Eppendorf	DNA LoBind Tubes, 2.0 ml* ThermoMixer® C SmartBlock™ 15 ml x 8 conical tubes	022431048 5382000015 5366000021

*No substitutions are allowed. Items have been validated by 10x Genomics® and are required for Chromium™ Genome workflow, training and system operations.

2.3. Preparation – Buffers

b) Prepare Lysis Buffer containing 10 mM Tris-HCl, 400 mM NaCl, and 2 mM EDTA, pH 8.0. 3 ml Lysis Buffer is needed for each sample extraction.

c) Prepare Proteinase K Solution containing 1 mg/ml Proteinase K, 1% SDS, and 2 mM EDTA, pH 8.0. 0.5 ml Proteinase K Solution is needed for each sample extraction.

3. Overnight Cell Lysis

NOTE

This Protocol was demonstrated using GM12878 suspension culture. The Protocol may be carried out using samples thawed after frozen storage. A Beckman Coulter Allegra® X-14R Centrifuge was used for step a.

a) Dispense 1.5 x 10^6 live cells per extraction into a 15 ml centrifuge tube. Centrifuge at **313 x g** for **7 min**.

b) Carefully remove the media, leaving only the cell pellet behind.

c) Add **3 ml** Lysis Buffer. Resuspend cell pellet by inverting 20 times.

d) Add **0.2 ml** 10% SDS.

e) Add **0.5 ml** Proteinase K Solution. Mix by inverting the tube 5 times.

f) Digest the cell lysate **overnight** (12 – 18 h) at **37°C** in a ThermoMixer® C.

4. DNA Purification

NOTE

A Beckman Coulter Allegra X-14R Centrifuge and a Eppendorf Centrifuge 5425R were used for steps b and f respectively.

a) Add **1.2 ml** 5M NaCl. Mix by inverting the tube 5 times.

b) Centrifuge at **4°C** and **1011 x g** for **15 min**.

c) Using a **wide-bore** pipette tip or a serological pipette, slowly transfer the supernatant containing the DNA to a new 15 ml tube and add **8 ml** absolute ethanol.

d) Gently rock the tube and look for presence of DNA strands.

e) Divide the DNA solution equally into six 2 ml Eppendorf DNA LoBind tubes.

NOTE

There is lower risk of losing the entire sample if the sample is divided during the following pelleting and resuspension steps.

f) Centrifuge at **4°C** and **6250 x g** for **5 min**.

g) Carefully remove the supernatant, leaving only the DNA pellet behind.

CRITICAL!

Know the expected position of the pellet, as it can be difficult to see.

h) Allow the pellets to air dry for **5 min**.

i) Using a **wide-bore** pipette tip, add **150 µl** TE Buffer to one tube and resuspend the DNA pellet with gentle pipette mixing, if necessary. Sequentially transfer the solution each to the remaining tubes to recombine all the DNA pellets into a single solution.

j) Allow the combined solution to homogenize at **room temperature** for **1 h**. Then store at **4°C** or proceed with the Chromium™ Genome Protocol.

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

5. Results

This Protocol was demonstrated using GM12878 suspension culture. All samples were analyzed via pulsed-field gel electrophoresis prior to sequencing. A subset of the resulting HMW gDNA from each sample was processed with Chromium™ Genome Reagent Kits and sequenced on an Illumina® HiSeq® 2500. The mean DNA size for each sample was >80 kb, which is the highest level of sample quality as defined in Technical Note *Sample Preparation Recommendations for the Chromium Genome Kit* (CG00045).