10x Genomics®

Sample Preparation Demonstrated Protocol

DNA Extraction from Single Insects

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Demonstrated Protocol

HMW gDNA Extraction

From Single Insects

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 increase sensitivity for large 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 a single *Drosophila melanogaster* larva, a single *Drosophila melanogaster* adult, and a single *Aedes aegypti* pupa. This Protocol can produce gDNA from ~48.5 kb – >200 kb from a single insect as 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 sample types, including sample age, transportation methods, type of sample, additives, freezing method, etc.

While this Protocol is demonstrated with *Drosophila melanogaster* and *Aedes aegypti*, it may be used for gDNA extraction from other types of insects. Modifications to this Protocol may be necessary for other sample types.

2. Getting Started

2.1. Tips & Safety

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 for 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.*

2.2. 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.

2.3. 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 Pellet Paint® Co-Precipitant	S6546 459836 69049
Thermo Fisher Sci	0.5M EDTA pH 8.0 10% SDS TE Buffer Fisherbrand™ RNase-Free Disposable Pellet Pestles *(If homogenizing by pestle)*	AM9260G 15553-027 12090-015 12-141-364
Eppendorf	DNA LoBind Tubes, 2.0 ml* ThermoMixer® C SmartBlock™ 2.0 ml x 24 Eppendorf tubes	022431048 5382000015 5366000035
-	Razor Blades *(If homogenizing by razor blades)*	-

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

2.4. Preparation – Buffers

a) Prepare Lysis Buffer containing 10 mM Tris-HCl, 400 mM NaCl, and 100 mM EDTA, pH 8.0. **600 µl** Lysis Buffer is needed for each sample.

b) Prepare Proteinase K Solution containing 1 mg/ml Proteinase K, 1% SDS, and 4 mM EDTA, pH 8.0. **100 µl** Proteinase K Solution is needed for each sample.

3. Homogenization & Overnight Lysis

A single insect can be homogenized by a pestle (Section 3.1) or by razor blades (Section 3.2). The pestle method is suitable for a small, low-chitinous insect. The razor blade method is suitable for a large and/or chitinous insect.

In this Protocol one low-chitin content *Drosophila melanogaster* larva was homogenized by the pestle method. A *Drosophila melanogaster* adult and an *Aedes aegypti* pupa were each homogenized by the razor blade method.

The Protocol may be carried out using sample thawed after frozen storage.

3.1. Homogenization by Pestle

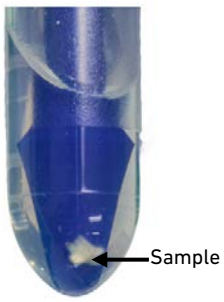
a) Mix **600 µl** Lysis Buffer, **40 µl** 10% SDS, and **100 µl** Proteinase K Solution in a 2 ml Eppendorf tube.

b) Using sterile forceps, fully immerse the insect in the solution.

c) Insert a sterile pestle in the tube and make contact with the insect.

d) Using a rolling motion of the pestle, apply pressure to grind the sample between the pestle head and the walls of the tube. Continue the rolling motion until there are no large clumps visible.

CRITICAL! *The insect should remain immersed in the solution during homogenization.*

Homogenization by Pestle	Homogenized Sample
	

e) Vortex for **5 sec**. Centrifuge briefly.

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

g) Proceed immediately with step 4 for DNA Purification.

3.2. Homogenization by Razor Blades

a) Mix **600 µl** Lysis Buffer, **40 µl** 10% SDS, and **100 µl** Proteinase K Solution in a 2 ml Eppendorf tube.

b) Using sterile forceps, immerse the sample in the solution. **Do not release** the insect from the forceps.

c) Place the wetted insect on a flat surface of a first razor blade positioned on a level surface.

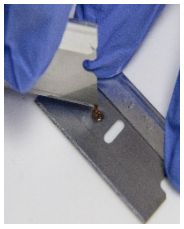
CRITICAL!

The insect should be in a small pool of solution while on the surface of the razor blade during homogenization.

d) Using a second razor blade, finely homogenize the sample on the surface of the first razor blade using a slicing motion. This may take up to **10 min**.

CRITICAL!

To minimize DNA degradation and maximize DNA yield, the sample should be finely homogenized.

Before Homogenization	Homogenization by Razor Blades	Homogenized Sample
		

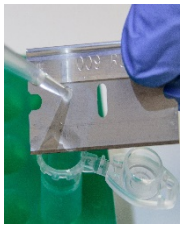
e) Using a **wide-bore** pipette tip, take **50 µl** solution from step 3.2a to rinse and collect the homogenized sample from the razor blade in the Eppendorf tube.

Repeat

f) Repeat step e until all the homogenized sample from the razor blade is collected in the tube.

CRITICAL!

To maximize DNA yield, all the homogenized sample should be collected from the razor blade surface.

g) Vortex for **5 sec**. Centrifuge briefly.

h) Digest the homogenized sample **overnight** (12 – 18 h) at **37°C** in a ThermoMixer® C. Proceed immediately with step 4 for DNA Purification.

4. DNA Purification

NOTE

An Eppendorf Centrifuge 5425R was used for steps b and f.

a) Add **240 µl** 5M NaCl to the tube containing the homogenized sample. Mix by inverting the tube 5 times.

b) Centrifuge at **4°C** at **1100 x g** for **15 min**.

c) Using a **wide-bore** pipette tip, slowly transfer the supernatant containing the DNA to a new 2 ml tube and add **1.2 ml** 100% ethanol.

d) Add **1 µl** Pellet Paint™ Co-Precipitant.

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

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

g) Carefully remove the supernatant, retaining the DNA pellet in the tube.

CRITICAL!

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

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

i) Using a **wide-bore** pipette tip, add **35 µl** TE Buffer and resuspend the DNA pellet with gentle pipette mixing.

j) Allow the solution to homogenize at **room temperature** for **1 h.**

k) Proceed with the Chromium Genome Protocol immediately or store the extracted gDNA at **4°C** for up to **2 weeks** or at **–20°C** for up to **6 months**.

5. Results

This Protocol was demonstrated using a single *Drosophila melanogaster* larva (homogenized by a pestle), and a single *Drosophila melanogaster* adult and a single *Aedes aegypti* pupa (each homogenized by razor blades). 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® NovaSeq®. The mean DNA size for each sample was 40 – 70 kb.