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Isolation of nuclei from mouse white adipose tissues for single-nucleus genomics --University of Minnesota TMCs

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ABSTRACT

This protocol describes the steps to isolate and obtain total nuclei from frozen mouse white adipose tissues (WAT). We utilized this protocol to obtain nuclei from 4- and 24- month-old mouse epididymal adipose samples. Additionally this protocol can be used for various other murine adipose depots, with adjustment of the final resuspension volume to accommodate variations in the size of the final nuclei pellet as a result of the nuclei yield.

Adapted from STAR Protocols - Isolation of nuclei from mouse white adipose tissues for single-nucleus genomics

Isolation of nuclei from mouse white adipose tissues for single-nucleus genomics.pdf

BEFORE START INSTRUCTIONS

General preparation
Timing: 1 h
1. Clean Dounce homogenizers and pre-cool them on ice.
CRITICAL: If nuclei are to be used for transcriptomics, rinse Dounce homogenizers with RNaseZAP and DEPC-treated water to minimize RNase activity.
2. Pre-cool centrifuges to 4C.
3. Pre-cool tubes for nuclei isolation and Petri dishes for tissue mincing on ice.
Note: For each isolation, prepare 13 50 mL tube, 23 5 mL DNA low binding tubes, 23 1.5 mL DNA low binding tubes, and 13 Petri dish.
4. Prepare nuclei isolation buffer (NIB) and nuclei resuspension buffer (NRB).
a. Filter buffers using a 0.2 mm syringe filter and pre-cool buffers on ice.
Note: Buffers are prepared freshly in order to minimize RNase activity.

Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides and recombinant proteins		
4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES)	Lonza	Cat#BE17-737E; CAS: 7365-45-9

Protocol status: Working
We use this protocol and it's working

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	REAGENT or RESOURCE	SOURCE	IDENTIFIER
	Bovine Serum Albumin	Sigma-Aldrich	Cat#B6917; CAS: 9048-46-8
	Diethyl dicarbonate (DEPC)	Sigma-Aldrich	Cat#D5758; CAS: 1609-47-8
	DL-Dithiothreitol (DTT)	New England Biolabs	Cat#B1034A; CAS: 3483-12-3
	IGEPAL CA-630	Sigma-Aldrich	Cat#I8896; CAS: 9002-93-1
	Magnesium chloride (MgCl2)	Sigma-Aldrich	Cat#M1028; CAS: 7786-30-3
	Phosphate buffered saline (PBS) (10×, pH 7.2)	Gibco	Cat#70013-016
	Potassium chloride (KCl)	Sigma-Aldrich	Cat#P9541; CAS: 7447-40-7
	RNase Inhibitor, Murine	New England Biolabs	Cat#M0314
	RNaseZAP	Sigma-Aldrich	Cat#R2020
	Sucrose	Sigma-Aldrich	Cat#S0389; CAS: 57-50-1
	Trypan Blue	Bio-Rad	Cat#1450013
	Biological Samples		
	Mouse white adipose tissues	N/A	N/A
	Critical commercial assays		
	Chromium Next GEM Chip G Single Cell Kit (48 rxns)	10x Genomics	Cat#1000120
	Chromium Next GEM Single Cell 3' Kit v3.1 (16 rxns)	10x Genomics	Cat#1000268
	Dual Index Kit TT Set A (for Gene Expression Libraries)	10x Genomics	Cat#1000215
	Experimental models: organisms/strains		
	Mus musculus C57BL/6J and C57BL/6N	N/A	N/A
	Other		
	0.2 µm Syringe filters	Sartorius	Cat#17845-ACK
	1.5 mL DNA LoBind tubes	Eppendorf	Cat#0030108051
	7 mL Dounce homogenizer	Sigma-Aldrich	Cat#D9063
	5 mL DNA LoBind tubes	Eppendorf	Cat#0030122348
	6 cm Petri dish	Thermo Scientific	Cat#150288
	50 mL Tube	SARSTEDT	Cat#62.547.254

	REAGENT or RESOURCE	SOURCE	IDENTIFIER
	10× Genomics Chromium Controller	10x Genomics	Cat#PN110203; RRID:SCR_019326
	BRAND counting chamber BLAUBRAND Bürker pattern	Sigma-Aldrich	Cat#BR718920
	Flowmi Cell Strainer, porosity 40 µm	Sigma-Aldrich	Cat#BAH136800040
	NovaSeq 6000 System	Illumina	Cat#20012850; RRID:SCR_016387
	pluriStrainer 70 µm (Cell Strainer)	pluriSelect	Cat#43-50070-51
	Surgical Scalpel Blade No.22	Swann-Morton	Cat#0208

Materials and equipment

Nuclei isolation buffer (NIB)

	Reagent	Final concentration	Amount
	Sucrose (0.5 M)	250 mM	500 µL
	HEPES (1 M)	10 mM	10 µL
	MgCl2 (150 mM)	1.5 mM	10 µL
	KCl (2 M)	10 mM	5 µL
	IGEPAL CA-630 (1%)	0.001%	1 µL
	DTT (0.1 M)	0.2 mM	2 µL
	RNase inhibitor (40,000 U/mL)	0.5 U/µL	12.5 µL
	DEPC-treated water	N/A	459.5 µL
	Total	**N/A**	**1 mL**

Nuclei resuspension buffer (NRB)

	Reagent	Final concentration	Amount
	BSA in 1× PBS (5%)	1%	200 µL
	MgCl2 (150 mM)	2 mM	13.3 µL
	RNase inhibitor (40,000 U/mL)	0.04 U/µL	1 µL
	PBS (1×)	N/A	785.7 µL

	Reagent	Final concentration	Amount
	Total	**N/A**	**1 mL**

Note

Note: NIB and NRB are prepared freshly, filtered, and kept on ice until use to minimize RNase activity. Sucrose is prepared freshly in DEPC-treated water. IGEPAL CA-630 solution is diluted in DEPC-treated water and can be stored for several months at 20°C–25°C. BSA is dissolved in 1× PBS (in DEPC-treated water) and can be stored at −20°C. 1× PBS solution is prepared by diluting a 10× PBS solution in DEPC-treated water.

Note

Alternatives: In principle, all reagents and resources listed in the key resources table can be replaced with equivalent items from other suppliers; however, the impact of alternative reagents on protocol performance has not been tested.

Tissue Homogenization

30m

1

Note

To minimize contamination with blood cells from the vasculature, it is recommended to do cardiac perfusion with 13 PBS in DEPC-treated water (typically 12 mL per perfusion) prior to isolating the tissue.

Transfer 400 mg of gonadal adipose tissue (or 100 mg of inguinal adipose tissue) to a pre-cooled Petri dish on ice (Figure 1A).

2 Add 500 µL of NIB and mince the tissue thoroughly (<1 mm3) using a scalpel (Figures 1B and 1C).

Note

CRITICAL: Nuclei yield will be reduced if the tissue is not finely minced.

3 With the scalpel, transfer the minced adipose tissue to a pre-cooled 7 mL glass Dounce homogenizer on ice (Figure 1D).

4 Homogenize the tissue to release nuclei by applying 5 strokes with pestle A (loose) followed by 5 strokes with pestle B (tight) (Figures 1E and 1F).

Note

CRITICAL: Homogenize carefully to minimize generation of heat and foam, which will impact nuclei intactness.

Note

Note: For adipose tissues from obese mice, use the same amount of tissue as for lean tissues, but homogenize in a total volume of 1 mL NIB and apply 10 strokes with pestle A followed by 5 strokes with pestle B.

5 Pre-wet a 70 µm cell strainer with DEPC-treated water.

6 Filter the homogenate through the pre-wetted cell strainer into a pre-cooled 50 mL tube on ice.

7 Wash the Dounce homogenizer with 1 mL NIB and filter through the same cell strainer.

8 Transfer the filtered homogenate to a pre-cooled 5 mL DNA Low binding tube on ice using a P1000 pipette.

9 Wash the cell strainer with 1 mL NIB and add to the filtered homogenate in the 5 mL tube to a total volume of ~2.5 mL using a P1000 pipette (Figure 1G).

Isolation of Nuclei

2h

10 Centrifuge the homogenate at 1000 x g for 10 min at 4°C using a fixed-angled rotor (Figure 2A).

Note

Nuclei are located as a smear along the side of the 5 mL tube.

11 Aspirate the lipid layer using a vacuum pump (Figure 2B).

Note

CRITICAL: Only remove the top lipid layer. Do not remove the entire supernatant, as this will lead to loss of nuclei.

12 Resuspend the pellet in the remaining supernatant using a P1000 pipette and transfer the resuspended nuclei to a new pre-cooled 5 mL DNA Low binding tube on ice.

13 To make sure that all nuclei are transferred, wash the first 5 mL tube twice with 1 mL NIB and transfer any remaining nuclei to the new 5 mL tube containing the resuspended nuclei using a P1000 pipette (Figure 2C).

14 Pellet the nuclei by centrifuging at 500 x g for 10 min at 4°C using a swing-bucket rotor (Figure 2D).

15 Aspirate the supernatant.

Note

CRITICAL: Be careful not to disturb the nuclei pellet.

15.1 Use vacuum aspiration to remove most of the supernatant (leave 80–100 mL supernatant) and remove remaining supernatant with a P100 pipette.

16 Wash the pellet - Resuspend the nuclei pellet in 750 µL of NIB

17 Pellet the nuclei by centrifuging at 500 x g for 10 min at 4°C

18 Resuspend the nuclei pellet in 100 µL NRB by pipetting using a regular P1000 pipette.

Note

For 2 yr old mice - pellet was resuspended in 750µL NRB.

19 Pre-wet a 40 mm tip strainer with NRB.

20 Filter the nuclei through the pre-wetted tip strainer using a P1000 pipette into a pre-cooled 1.5 mL DNA Low binding tube on ice (Figure 2E) to remove any remaining debris and avoid nuclei aggregation.

Note

The nuclei concentration will decrease by 30%–40% following filtration.

21 Proceed to nuclei counting using a Bürker counting chamber and Trypan Blue.

21.1 Mix nuclei and Trypan Blue in equal ratio in a separate tube.

21.2 Transfer 10 µL of the nuclei-Trypan Blue solution to the Bürker counting chamber.

21.3 Count at least 3 squares using bright-field microscopy and quantify the nuclei concentration.

Note

If nuclei are diluted while counting, multiply the equation for calculating the concentration of nuclei with the dilution factor.

Note

CRITICAL: Verify that nuclei are mostly intact and not clumping (Figures 3A and 3B). Note, that intact nuclei isolated from adipose tissues have a heterogenic morphology.