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Nuclei Isolation from Human Frozen Endometrium with Sample Multiplexing for 10X Genomics Multiome (ATAC + Gene Expression) Assay

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We use this protocol and it's working

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Abstract

This protocol was developed to enable the isolation of nuclei from human frozen endometrium suitable for the 10X Genomics Multiome (ATAC + Gene Expression) assay. Nuclei are prepared by mechanical homogenization of the tissue under mild cell lysis conditions, followed by washing steps. To reduce technical variability and costs, samples are multiplexed by hashing the nuclei of each sample with a unique oligonucleotide-tagged antibody against the nuclear pore complex. Hashed nuclei samples are pooled together at equal ratio and the resulting pool is permeabilized. Sample multiplexing also allows super-loading, however, it does not decrease the cost of sequencing. Prior to loading onto the Chromium X, the nuclei pool is transposed as per the Multiome (10X Genomics) protocol.

Guidelines

- This protocol has been optimized for non-tumor human frozen endometrium tissue. The human endometrium tissue is highly heterogeneous and therefore NP-40 concentration may need to be adapted to the tissue/sample condition. Whenever possible, performing a quick test on the tissue is very important to ensure good nuclei integrity. For the majority of samples tested with this protocol, 0.067% NP-40 was the optimal concentration.
- For a different tissue type, it is recommended to perform a trial on test samples to adjust parameters such as mincing time, lysis agent concentration, lysis time, mechanical homogenization method, and assess hashing feasibility.
- Always inspect the nuclei integrity under the microscope. Nuclei with good integrity are intact and their nucleoli may even be well visible. If nuclei clumping is observed, it is normally a sign of nuclei bursting due to over-lysis.
- Nuclei pooling takes place just before the permeabilization step and after hashing each sample independently with a unique HTO.
- In this protocol, a nuclei pool comprises 2 million nuclei corresponding to 0.5 million nuclei from each of the 4 hashed samples pooled together.
- Multiplexing a maximum of 4 samples is recommended. Nuclei hashing of more than 4 samples for a Multiome assay may result in less than 5000 nuclei recovered per sample after Seurat analysis.

Materials

REAGENTS AND CONSUMABLES

	Item	Vendor	Catalog #
	1.5mL DNA LoBind Tubes	Eppendorf	022431021
	2mL DNA LoBind Tubes	Eppendorf	022431048
	Acridine Orange/Propidium Iodide Stain	Logos Biosystems	F23001
	Bel-Art Flowmi Cell Strainers (40 µm)	Fisher Scientific	14-100-150
	Calcium Chloride	VWR	97062-590
	Cell Staining Buffer	BioLegend	420201
	CELLTRICS 20 µm filters	Fisher Scientific	NC9699018
	Chromium Next GEM Single Cell Multiome ATAC + Gene Expression Reagent Bundle, 16 rxns	10X Genomics	1000283
	Digitonin (5%)	Invitrogen	BN2006
	Disposable Scalpels, Sterile, Sklar	VWR	82029-850
	DTT Solution (1M)	Tribioscience	TBS5039
	EZFlow Filter Unit, Foxx PES Membrane 0.22µm Pore Size, Sterile	Foxx Life Sciences	371-2215-OEM
	Human TruStain FcX (Fc Receptor Blocking Solution)	BioLegend	422302
	KIMBLE Dounce tissue grinder set	Sigma-Aldrich	D8938-1SET
	LUNA-FX7 Automated Cell Counter	Logos Biosystems	L70001
	MACS BSA Stock Solution	Miltenyi Biotec	130-091-376
	MACS SmartStrainers (30 µm)	Miltenyi Biotec	130-098-458
	Magnesium Acetate Tetrahydrate	VWR	97061-060
	Magnesium Chloride Solution (1M)	Sigma-Aldrich	M1028
	Molecular Grade Water (Nuclease-free Water)	G-Biosciences	786-292
	Nonidet P 40 Substitute (NP-40)	Sigma-Aldrich	74385
	PhotonSlide, 50 Slides	Logos Biosystems	L12005
	Pipette Tips RT LTS 1000µL FLW 768A/8 (Wide bore tips)	Mettler Toledo	30389218

	Item	Vendor	Catalog #
	Polysorbate 20 (Tween-20)	VWR	97062-332
	Protector RNase Inhibitor	Roche	3335399001
	Recombinant RNase Inhibitor	Takara	2313B
	RNaseZap RNase Decontamination Solution	Thermo Fisher Scientific	AM9780
	Sodium Chloride Solution (5M)	Sigma-Aldrich	71386-1L
	SP Bel-Art Disposable Polypropylene Pestles	VWR	47750-354
	Sucrose	VWR	470302-810
	TotalSeq-A anti-Nuclear Pore Complex Proteins Hashtag Antibodies (HTO)	BioLegend	Varied
	Trizma hydrochloride solution (pH 7.4, 1M)	Sigma-Aldrich	T2194-1L
	Trypan Blue Solution, 0.4%	Thermo Fisher Scientific	15250061

STOCK BUFFERS

- All stock buffers should be kept at 4°C for a maximum of 6 months.
- To all buffers add only right before use: 1mM DTT and 1U/µL RNase Inhibitor.
- Transposition mix should only be prepared when ready for the transposition step.
- Prepare an NP-40 stock solution of 10%.
- The volumes indicated for the stock buffers are sufficient for 4 samples or 1 pool.

Homogenization Suspension Buffer for Lysis (HSB-1) – 15 mL

	Reagents	Volume (µL)	Final Concentration
	Sucrose (1M)	4800	320mM
	Tris-HCl pH 7.4 (1M)	150	10mM
	Calcium Chloride (1M)	45	3mM
	Magnesium Acetate Tetrahydrate (1M)	45	3mM
	DTT Solution (1M)	15	1mM
	Protector RNase Inhibitor (40U/µL), Roche	375	1U/µL
	Nuclease-free Water	9570	NA

	Reagents	Volume (µL)	Final Concentration
	Total Volume	15000	NA

Homogenization Suspension Buffer for Washes (HSB-2) – 25 mL

	Reagents	Volume (µL)	Final Concentration
	Sucrose (1M)	8000	320mM
	Tris-HCl pH 7.4 (1M)	250	10mM
	Calcium Chloride (1M)	75	3mM
	Magnesium Acetate Tetrahydrate (1M)	75	3mM
	DTT Solution (1M)	25	1mM
	Recombinant Rnase Inhibitor (40U/µL), Takara	625	1U/µL
	Nuclease-free Water	15950	NA
	Total Volume	25000	NA

Note

- For tissue lysis steps use HSB-1 (Protector RNase Inhibitor, Roche).
- For quenching-wash steps use HSB-2 (Recombinant RNase Inhibitor, Takara).
- Filter the HSB buffers using a 0.22 µm PVDF filter unit.

Nuclei Staining Buffer for Incubation (NSB-1) – 2 mL

	Reagents	Volume (µL)	Final Concentration
	10% Tween-20	2	0.01%
	Calcium Chloride (1M)	1.8	0.9mM
	Magnesium Chloride (1M)	1	0.5mM
	DTT Solution (1M)	2	1mM
	Protector RNase Inhibitor (40U/µL), Roche	50	1U/µL
	Cell Staining Buffer - BioLegend	1943.2	1X
	Total Volume	2000	NA

Nuclei Staining Buffer for Washes (NSB-2) – 20 mL

	Reagents	Volume (µL)	Final Concentration
	10% Tween-20	20	0.01%
	Calcium Chloride (1M)	18	0.9mM
	Magnesium Chloride (1M)	10	0.5mM
	DTT Solution (1M)	20	1mM
	Recombinant RNase Inhibitor (40U/µL), Takara	500	1U/µL
	Cell Staining Buffer - BioLegend	19432	1X
	Total Volume	20000	NA

Note

- For hashtag antibody incubation step use NSB-1 (Protector RNase Inhibitor, Roche).
- For hashing washing steps use NSB-2 (Recombinant RNase Inhibitor, Takara).

Permeabilization Wash Buffer (PWB) – 3 mL

	Reagents	Volume (µL)	Final Concentration
	Tris-HCl pH 7.4 (1M)	30	10mM
	Sodium Chloride (5M)	6	10mM
	Magnesium Chloride (1M)	9	3mM
	10% Tween-20	3	0.01%
	MACS BSA Stock Solution	300	1%
	DTT Solution (1M)	3	1mM
	Protector RNase Inhibitor (40U/µL), Roche	75	1U/uL
	Nuclease-free Water	2574	1X
	Total Volume	3000	NA

Permeabilization Digitonin Buffer (PDB) – 0.5 mL

	Reagents	Volume (µL)	Final Concentration
	10% NP-40	0.75	0.015%
	0.5% Digitonin	1	0.001%
	PWB	498.25	1X
	Total Volume	500	NA

Note

- There is no need to add RNase Inhibitor in the PDB buffer as this was added already in PWB.

Nuclei Buffer (NB) – 1 mL

	Reagents	Volume (µL)	Final Concentration
	20X Nuclei Buffer (10X Genomics)	50	1X
	DTT Solution (1M)	1	1mM
	Protector RNase Inhibitor (40U/µL), Roche	25	1U/uL
	Nuclease-free Water	924	1X
	Total Volume	1000	NA

Troubleshooting

Before start

- All steps should be performed on ice or at 4°C.
- All reagents should be kept at 4°C.
- Pre-chill a swinging bucket centrifuge.
- Pre-chill all douncers, pestles, tubes, petri dishes, scalpels and forceps.
- Spray down all work surfaces with RNaseZap.
- Use disposable, RNase-free pipettes tips and tubes.
- Use wide bore tips where indicated.
- For each sample the following will be required: 1X glass douncer and respective glass pestle (only pestle A – loose pestle is needed), 1X petri dish, 1X scalpel, 1X forceps, 1X 15mL falcon tube fitted with a MACS SmartStrainer (30 µm), 3X 1.5mL Eppendorf DNA LoBind Tubes, 1X disposable polypropylene pestle.
- Remove samples from liquid nitrogen or -80°C storage and keep them on dry ice until use.
- Use 30-50mg of tissue per sample.

Tissue Homogenization and Nuclei Isolation

1 Place 30-50mg of frozen tissue on a pre-chilled petri dish and immediately add 150 µL of HSB-1 to cover the tissue.

2 On ice, quickly mince/chop the tissue into 2-3mm sized pieces.

3 Transfer the tissue pieces/HSB mix to a douncer. Rinse the petri dish with 450 µL of HSB-1 and transfer it to the same douncer. The total volume of HSB-1 in the douncer will be 600 µL .

4 Add 4 µL of 10% NP-40 (0.067% NP-40 final concentration) to the douncer and start the timer pre-set for 00:05:00 to initiate lysis incubation step.

5 Gently homogenize the sample using the loose pestle (pestle A) by stroking 15 times. Keep the douncer on ice at all times and avoid making bubbles.

6 Half way through the lysis incubation time, gently pipette mix 5 times the tissue lysate with a wide bore 1mL tip.

7 At the end of the 5 minute lysis incubation time, quench the lysate with 1 mL of HSB-1 and gently pipette mix 10 times with a wide bore 1mL tip.

8 Filter the homogenate through the 30 µm MACS SmartStrainer fitted into a 15mL falcon tube.

9 Rinse the douncer with 1 mL HSB-2 to recover any remaining tissue homogenate and filter it through the same MACS SmartStrainer.

10 Rinse the MACS SmartStrainer with an additional 2 mL of HSB-2 to force all nuclei to pass through the strainer.

11 Using forceps or a 1mL wide bore tip, quickly recover the bigger tissue pieces left on the strainer and transfer them to a 1.5mL Eppendorf tube. Immediately add 200 µL of

HSB-1.

12 Very gently homogenize the tissue pieces 5 times with a disposable polypropylene pestle.

13 Rinse the disposable pestle with 300 µL of HSB-1 into the same Eppendorf tube containing the lysate.

14 Add 2 µL of 10% NP-40 (0.04% NP-40 final concentration) and start the timer pre-set for 00:03:00. Gently pipette mix 10 times with a wide bore 1mL tip.

15 At the end of the 3 minute lysis incubation time, quench the lysate with 1 mL of HSB-1 and gently pipette mix 10 times with a 1mL wide bore tip.

16 Filter the homogenate through the same 30 µm MACS SmartStrainer fitted into a 15mL falcon tube from step 8.

17 Rinse the Eppendorf tube with 1 mL of HSB-2 to recover any remaining tissue homogenate and filter it through the MACS SmartStrainer.

18 Rinse the MACS SmartStrainer with an additional 2 mL of HSB-2.

19 Centrifuge the nuclei at 500 x g, 4°C, 00:05:00.

20 Remove as much supernatant as possible without disturbing the nuclei pellet. Gently add 200 µL of NSB-1 without disturbing the pellet. Let it sit on ice for 00:05:00.

21 After the incubation time, gently pipette mix at least 10 times to resuspend the nuclei pellet.

22 Count the nuclei using the AO/PI dye and an automatic cell counter (e.g. LUNA-FX7™ Automated Cell Counter).

Nuclei Hashing and Pooling

23 Transfer 1.5 million nuclei into a new 1.5mL Eppendorf tube. Bring the total volume up to 150 µL by adding appropriate amount of NSB-1 (Nuclei concentration: 1.5 million/150µL).

Note

- If volume is greater than 150µL, centrifuge at 500 x g, 4°C, 00:05:00 . Remove as much supernatant as possible. Gently resuspend the nuclei pellet with 150 µL of NSB-1. Ensure the pellet is fully resuspended and the nuclei suspension is homogenous.
- In the case where a lower number of nuclei are recovered, you may proceed with the hashing step by adjusting the quantity of HTO as described in step 25 below.

24 Add 5 µL of Human TruStain FcX blocking solution to each nuclei sample, pipette mix 5 times and incubate on ice for 00:10:00 .

25 After the blocking step, add to each sample 1.5 µg (3 µL) of a unique hashtag antibody (Hashing concentration: 1.5 million nuclei/1.5µg HTO/150µL). Pipette mix 10 times.

Note

- Make note of which HTO was added to which sample. This is very important for downstream analysis and sample identification within a pool.
- Hashing concentration recommendations: 0.5-1 million nuclei/1µg HTO/100µL or 1.5 million nuclei/1.5µg HTO/150µL.

26 Incubate on ice for 00:30:00 .

27 Wash 1: After hashing incubation add 900 µL of NSB-2 to each sample.

28 Centrifuge at 500 x g, 4°C, 00:05:00 .

29 Carefully remove the supernatant, leaving behind just enough volume to cover the pellet.

30 Wash 2: Add 900 µL of NSB-2 to each sample. Gently pipette mix 5 times.

31 Centrifuge at 500 x g, 4°C, 00:05:00 .

32 Carefully remove the supernatant leaving behind just enough volume to cover the pellet.

33 Wash 3: Add 900 µL of NSB-2 to each sample. Gently pipette mix 5 times.

34 Centrifuge again at 500 x g, 4°C, 00:05:00 .

35 Carefully remove the supernatant without disturbing the pellet. Remove as much buffer as possible to reduce antibody carryover.

36 Resuspend the nuclei in 500 µL of NSB-2 by gently pipette mixing at least 10 times or until no aggregates are visible by eye.

37 Assess the nuclei suspension by checking for the presence of nuclei clumps or aggregates through trypan blue staining.

Note

- If aggregates/clumps are present, further pipette mix 5 times the nuclei suspension and filter through a CELLTRICS 20 µm filter fitted in a 1.5mL Eppendorf tube on ice. Rinse the filter with 500µL of NSB-2 to push all the nuclei through the filter.

38 Gently pipette mix the nuclei suspension and count the nuclei as described in step 22.

39 In a new 2mL Eppendorf tube add 0.5 million nuclei from each hashed sample (maximum of 4 samples or 2 million nuclei). Gently pipette mix 5 times.

Note

- It is very important to pool the nuclei from each sample at equal proportions. If one sample has a total of less than 0.5 million nuclei, use the same nuclei number for the other 3 samples.

40 Centrifuge at 500 x g, 4°C, 00:05:00 .

Nuclei Permeabilization and Transposition

41 Remove as much supernatant as possible and gently add 500 µL of PWB without disturbing the pellet. Incubate on ice for 00:05:00 .

42 After the incubation time, very gently resuspend the nuclei pellet by pipette mixing 5 times.

43 Centrifuge the nuclei suspension at 500 x g, 4°C, 00:05:00 .

44 Remove completely the supernatant.

45 Carefully add 125 µL of PDB and start the timer pre-set to 00:02:00 . Gently pipette mix the nuclei 10 times and incubate the nuclei on ice.

46 After the incubation time, add 1 mL of PWB and pipette mix 3 times.

47 Centrifuge at 500 x g, 4°C, 00:05:00 .

48 Resuspend the nuclei in 125 µL of NB by gently pipette mixing at least 10 times.

49 Count the nuclei as described in step 22.

50 If nuclei aggregates or clumps are present, filter the nuclei pool through a 40 µm Flowmi Cell Strainer.

51 Count the nuclei again and aim for a concentration of 8-8.5 million nuclei/mL (4-4.25×10^4 nuclei in 5µL).

Note

- If nuclei concentration is more dilute than the required, proceed to centrifugation at 500 x g, 4°C, 00:05:00 to concentrate the nuclei suspension. Always consider a 50% loss of nuclei with each centrifugation post-permeabilization step.

52 Prepare the transposition mix as described in the 10X Genomics Multiome protocol. Aim to load 4-4.25×10^4 nuclei for a total volume of 5µL.

53 Proceed as per 10X Genomics Multiome protocol.

Protocol references

1. “Nuclei isolation from fresh and frozen brain tissue”, Schmutz et al., protocols.io, **dx.doi.org/10.17504/protocols.io.bvhhn336**.
2. “Protocol for nuclei isolation from fresh and frozen tissues for parallel snRNA-Seq and snATAC-Seq on 10x Chromiumium platform using the same nuclei preparation (UPDATED version) V.4”, Luciano G. Martelotto, protocols.io, **dx.doi.org/10.17504/protocols.io.bdeni3de**.
3. “Chromium Next GEM Single Cell Multiome ATAC + Gene Expression Reagent Kits User Guide”, CG000338 Rev G, 10X Genomics