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Nuclei isolation from fresh and frozen brain tissue - for single nucleus RNAseq or 10x Multiome

Isabelle Schmutz[1], Jade Carter[1]

[1]New York Genome Center

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New York Genome Center

Isabelle Schmutz
New York Genome Center

ABSTRACT

This protocol is for nuclei isolation from frozen mouse brain or human postmortem brain and processing of these nuclei for loading on single cell sequencing platforms. Nuclei are prepared by dounce homogenization under mild cell lysis conditions. They are purified from myelin, subcellular debris and ambient RNA using centrifugation through an iodixanol cushion. Isolated nuclei can be directly loaded onto a single nucleus sequencing platform or they can be additionally permeabilized for combined single nucleus RNAseq/ATACseq.

The protocol is compatible with working on small amounts of tissue, using microtubes and benchtop centrifuges. We have successfully prepared nuclei from as little as 10 mg of frozen mouse brain tissue and processed them through the 10x Genomics Multiome assay.

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Nuclei isolation

1. Start with 10-100 mg of frozen tissue sample, keep sample on dry ice until used.
2. Prepare a glass dounce homogenizer, add 500 µl of homogenization buffer and 5 µl of 10% NP-40 (final 0.1% NP-40) and keep on ice.
3. Add tissue sample to the homogenizer and homogenize by douncing 15 times with pestle A (loose) followed by 15 times with pestle B (tight).
4. Transfer homogenate to 1.5 ml microcentrifuge tube and incubate for 5 min on ice.
5. Add 500 µl homogenization buffer (without NP-40) to stop cell lysis. Pipet mix and filter through 70 µm FlowMi.
6. Spin down nuclei, 500g for 5 min at 4°C. If possible use a swing-bucket rotor for this centrifugation to maximize the number of pelleted nuclei.
7. Remove supernatant and resuspend nuclei in 750 µl homogenization buffer.
8. Dilute the homogenate with 750 µl of 50% iodixanol to adjust the concentration to 25% iodixanol/0.16 M Sucrose.
9. Prepare a 2 ml microcentrifuge tube. Add 500 µl of 29% iodixanol (this will be the iodixanol cushion).
10. Carefully overlay the iodixanol cushion with the homogenate, making sure not to mix the 29% cushion and the 25% iodixanol homogenate (pipet the homogenate slowly onto the side of the tube)
11. Spin down, 6000g for 30 min at 4°C. We used a fixed angle rotor for this step.
12. After centrifugation, purified nuclei should be visible as a pellet on the side of the tube and white matter/debris should float on top.
13. Using a vacuum, carefully aspirate the debris together with the supernatant. Remove as much supernatant as possible without disturbing the nuclei pellet.
14. Resuspend the nuclei pellet in 125 µl PBS/1% BSA. Pipet mix for 20-40x.
15. Count nuclei. The number of nuclei recovered depends on the region of interest and the size of the tissue sample used. After centrifugation through the iodixanol cushion expect to recover 50-70% of the nuclei used as input.
16. At this step nuclei are ready to be loaded on a single nucleus RNAseq platform.

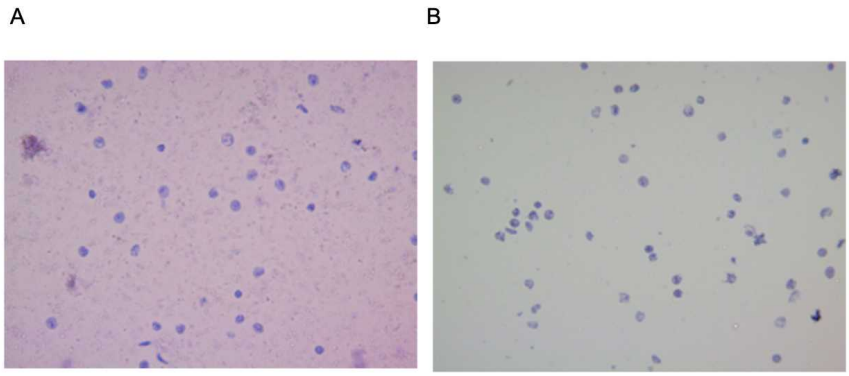

Figure 1. Trypan Blue Staining of nuclei from mouse brain in homogenate (A) and after iodixanol cushion purification (B).

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Additional processing for combined single nucleus RNAseq/ATACseq (10x Multiome)

Nuclei permeabilization (to make the nuclear membrane permeable for Tn5 transposase)

1. Spin down nuclei, 500g for 5 min at 4°C. If possible use a swing-bucket rotor to maximize the number of recovered nuclei.
2. Remove supernatant and resuspend nuclei in 100 µl lysis buffer.
3. Incubate for exactly 2 min on ice.
4. Add 1000 µl wash buffer.
5. Spin down, 500g for 5 min at 4°C (swing bucket rotor)
6. Resuspend nuclei in 100-200 µl of 1x Nuclei buffer (10x Genomics). Pipet mix for 20-40x.
7. Count nuclei
8. At this step nuclei are ready to be loaded on the combined single nucleus RNAseq/ATACseq platform (10x Multiome assay).

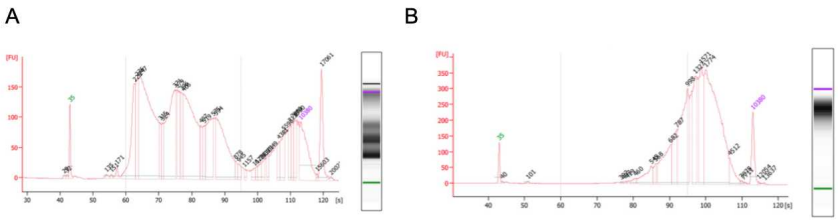

Figure 2: Bioanalyzer traces for ATAC fragments (A) and cDNA (B) prepared from mouse brain nuclei that were processed with the 10x multiome assay. Nuclei were prepared from 15 mg of fresh frozen mouse brain tissue.

Buffers

Homogenization buffer

10 mM Tris-HCl pH7.4
320 mM Sucrose
3 mM $CaCl_2$
3 mM $Mg(Ac)_2$
Add before use: 1 mM DTT, 1 U/µl RNAse inhibitor

10% NP-40

50% iodixanol

10 mM Tris-HCl pH7.4
3 mM $CaCl_2$
3 mM $Mg(Ac)_2$
Optiprep/Iodixanol 50%
Add before use: 1 mM DTT, 0.8 U/µl RNase inhibitor

29% iodixanol

10 mM Tris-HCl pH7.4
3 mM $CaCl_2$
3 mM $Mg(Ac)_2$
10 mM Tris-HCl pH7.4
0.16 M Sucrose
Optiprep/Iodixanol 29%
Add before use: 1 mM DTT, 0.5 U/µl RNase inhibitor

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1x PBS/1% BSA
add before use: 1 mM DTT, 1 U/µl RNAse inhibitor

Lysis buffer(as of 10x demonstrated protocol, 0.1x)
10 mM Tris-HCl pH7.4
10 mM NaCl
3 mM $MgCl_2$
1% BSA,
0.01% Tween 20
0.01% NP-40
0.001% Digitonin (1:10)
Add before use: 1 mM DTT, 1 U/µl RNase inhibitor

Wash buffer
10 mM Tris-HCl pH7.4
10 mM NaCl
3 mM $MgCl_2$
1% BSA
0.01% Tween 20
Add before use: 1 mM DTT, 0.5U/µl RNase inhibitor

1x Nuclei buffer
dilute from 20x stock
Add before use: 1 mM DTT, 0.5U/µl RNase inhibitor

RNase inhibitor: Protector - Sigma/Millipore (3335399001)

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