PARSE BIOSCIENCES
CUSTOMER DEVELOPED PROTOCOL

Single nuclei isolation from OCT-embedded adult rat auditory cortex


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About the CLEF Lab

The CLEF (Cortex, Learning, Epigenetics, & Function) Laboratory is a part of the Department of Psychology at Rutgers University. Their goal is to understand how the adult brain learns and remembers information from our daily and significant experiences. A core feature of their investigation is understanding the mechanisms of neuroplasticity, the brain's ability to rewire and reorganize, especially in the cortex, at the highest neural levels of representation.

This protocol was used in

Gungor Aydin, A., Lemenze, A. & Bieszczad, K.M. Functional diversities within neurons and astrocytes in the adult rat auditory cortex revealed by single-nucleus RNA sequencing. Sci Rep 14, 25314 (2024). https://doi.org/10.1038/s41598-024-74732-7

Materials

Material	Supplier	Part Number	Notes
2-methyl-butane (Isopentane)	Sigma-Aldrich®	277258	or equivalent
Optimal cutting temperature medium (OCT)	Sakura Finetek or equivalent	4583	or equivalent
Tris-HCl	Millipore Sigma®	T2194	or equivalent
NaCl	Millipore Sigma	59222C	or equivalent
$MgCl_2$	Millipore Sigma	M1028	or equivalent
Nonidet® P40 (NP-40)	Millipore Sigma	74385	or equivalent
Nuclease-free Water	Thermo Fisher Scientific®	AM9922	or equivalent
PBS, pH 7.4	Gibco®	10-010-031	or equivalent
BSA	Thermo Fisher Scientific	AM2616	or equivalent

Centrifuge with swinging bucket rotor	Varies	Varies	Compatible with 15 mL or 5 mL centrifuge tubes and capable of reaching 4°C.
1.5 ml microcentrifuge tubes	Fisherbrand®	05-408-130	or equivalent
1.5 ml Protein LoBind®	Eppendorf®	022431081	or equivalent
Plastic pestle homogenizer	Fisherbrand	12-141-364	or equivalent
30 µm strainer	pluriSelect®	435003003	or equivalent
RNaseZAP® RNase Decontamination Solution	Thermo Fisher Scientific	AM9780	
Nuclei Isolation Column			

Lysis Buffer

Reagents	Stock Concentration	Final Concentration	Volumes for 10 mL
Tris-HCl	10 mM	10 mM	100 µl
NaCl	5 M	10 mM	20 µl
$MgCl_2$	1 M	3 mM	30 µl
Nonidet P40 (NP-40)	10%	0.1%	100 µl
Nuclease-free Water	-	-	9.75 mL

Nuclei Wash and Resuspension Buffer

Reagents	Stock Concentration	Final Concentration	Volumes for 5 mL
PBS	1X	-	3.975 mL
BSA	5%	1%	1 mL
0.2 U/µl RNase inhibitor	40X	0.2X	25 µl

Preparation

1. Clean the bench top and dissection area with 70% ethanol, followed by RNaseZAP to remove RNases.
2. Tissue Collection
    a. Rapidly remove rat brains and flash-freeze in pre-chilled 2-methylbutane (dry ice or liquid nitrogen) for 60 seconds, then store at -80°C until sectioning.

b. Embed brains in optimal cutting temperature medium (OCT) and section with a cryostat (250 μm sections).
c. Micropunch sections containing the auditory cortex (AC) with a 1 mm diameter micropuncher.
d. Transfer to ice-cold centrifuge tubes and store at -80°C until nuclei isolation.

Procedure

Note: Keep all tools, materials, and reagents on ice. Pre-cool the swinging bucket centrifuge to 4°C

1. Combine frozen micropunches and briefly thaw on wet ice.
2. Transfer to a 1.5 mL tube with 400 μl chilled lysis buffer.
3. Dissociate the tissue on ice using a plastic pestle homogenizer to grind the tissue manually until homogeneous.
4. Incubate on ice for 10 minutes.
5. Pass micropunches through pre-chilled Nuclei Isolation Column* (spin column) by centrifuging at 500 x g for 3 minutes at 4°C in a swinging bucket centrifuge to pellet nuclei.
6. Remove the supernatant without disrupting the pellet.
7. Wash nuclei-pellets with 1 mL chilled Nuclei Wash and Resuspension Buffer by gently pipette mixing 10 times.
8. Filter nuclei through a 30 μm strainer into a 1.5 mL centrifuge tube (Protein LoBind tube highly recommended)
9. Centrifuge at 500 x g for 3 minutes at 4°C in a swinging bucket centrifuge to pellet nuclei.
10. Remove the supernatant without disrupting the pellet.

11. Resuspend nuclei in 500 µL Nuclei Wash and Resuspension Buffer by gently pipetting 10 times.
12. Filter twice through a 30 µm strainer, collecting the filtrate in a new tube each time.
13. Count nuclei using a preferred accurate method.

Note: In our experience, nuclei are much stickier than whole cells. For researchers making the single-nuclei suspension for the first time, we suggest confirming that the sample contains mostly single nuclei. To do this, take 10 µL of the sample and stain it with Hoechst, DAPI, or AOPI for more than 5 min, place it on a hemocytometer, and visualize it under a 20X and 40X objective on an epifluorescence microscope. A best practices resource for hemocytometer use, see: https://www.hemocytometer.org/

14. Aliquot nuclei and centrifuge at 200 x g for 10 min at 4 °C.
15. Proceed to the appropriate Parse Biosciences Nuclei Fixation Kit User Guide (either standard Nuclei Fixation, Low-Input Nuclei Fixation).

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