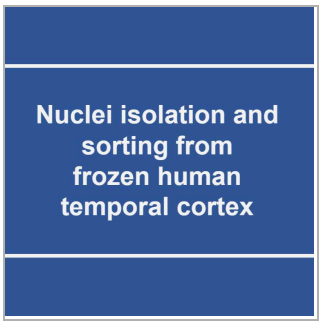

NOV 21, 2022

WORKS FOR ME 1

Nuclei Isolation and Sorting from Frozen Human Temporal Cortex

COMMENTS 0

DOI
dx.doi.org/10.17504/protocols.io.j8nlk4k55g5r/v1

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Daniel's workspace

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ABSTRACT

This protocol is about nuclei isolation and sorting from frozen human temporal cortex.

ATTACHMENTS

Scherzer_Neurogenomics_Laboratory-_Nuclei_isolation_and_sorting_from_frozen_human_temporal_cortex.pdf

DOI

dx.doi.org/10.17504/protocols.io.j8nlk4k55g5r/v1

PROTOCOL CITATION

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KEYWORDS

nuclei isolation, frozen human temporal cortex, ASAPCRN

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OWNERSHIP HISTORY

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Oct 03, 2022	Daniel El Kodsi

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MATERIALS TEXT

1. Dounce tissue grinder set **Sigma Aldrich Catalog #D8938-1SET**
2. Nuclei Isolation Kit: Nuclei PURE Prep **Sigma Aldrich Catalog #NUC201-1KT**
3. DTT **Sigma Aldrich Catalog #43816-10ML**
4. DPBS with no calcium and magnesium **Thermo Fisher Scientific Catalog #14190-144**
5. UltraPure™ BSA (50 mg/mL) **Thermo Fisher Scientific Catalog # AM2616**
6. Recombinant RNase Inhibitor **Clontech Catalog #2313B**
7. 70 µm Sterile Cell Strainer **Fisher Scientific Catalog #22363548**
8. Corning™ Falcon™ Test Tube with 35µm Cell Strainer Snap Cap **Corning Catalog #352235**
9. ART™ Wide Bore Filtered Pipette Tips **Thermo Fisher Scientific Catalog #2079G**

Buffer (For 2 samples):

1. Lysis buffer (LB):
   - 8 mL Nuclei PURE Lysis buffer
   - 8 µL DTT
   - 80 µL 10% Triton X-100
2. Nuclei wash and resuspension buffer (NWRB):
   - 14.8 mL DPBS
   - 150 µL BSA
   - 75 µL RNase Inhibitor (40 U/ml)
3. Nuclei wash and resuspension buffer with DAPI (NWRBD):
   - 5.5 mL NWRB
   - 11 µL DAPI (5 mg/ µl)

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

BEFORE STARTING

Prepare Buffers as described in section 'Materials'.

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ATTACHMENTS

Scherzer_Neurogenomics_Laboratory-_Nuclei_isolation_and_sorting_from_frozen_human_temporal_cortex.pdf

40m

Method

1 Cut 100 mg - 200 mg frozen temporal cortex tissue into small pieces on dry ice.

2 Put cut tissue into a microcentrifuge tube (pre-cooled on dry ice), weigh in a balance.

3 Transfer tissue to a glass dounce tissue grinder on regular ice.

4 Add 2 mL LB to the tissue grinder.

5 Homogenize tissue with pastel A *10 times* and pastel B *10 times*.

6 Transfer the homogenate into a 2 ml microcentrifuge tube.

5m

7 Incubate On ice for 00:05:00, mix with Wide bore tips once during the incubation.

8 Filter the homogenate with a 70 µmSstrainer.

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

10 Remove the supernatant and resuspend the pellet in 1.5 mL LB with Wide bore tips.

11 Incubate On ice for 00:05:00 . 5m

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

13 Remove the supernatant and add 500 µL NWRB to the pellet without resuspension.

14 Incubate On ice for 00:05:00 . 5m

15 Add 1.0 mL additional NWRB to the tube and resuspend the pellet.

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

17 Remove the supernatant and resuspend the pellet in 1.8 mL NWRB .

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18 Pipette 200 µL nuclei from original tube (T1) into a new tube (T2).

19 Centrifuge both T1 and T2 at 500 x g, 4°C, 00:05:00 .

20 Remove supernatant in T1 and resuspend pellet with 500 µL NWRBD .

21 Remove supernatant in T2 and resuspend pellet with 65 µL NWRB .

22 Filter nuclei in T1 and T2 with 35 µm strainer respectively.

23 Check nuclei under microscope and count the number with cell counter *(Optional)*.

24 Add 2 mL NWRBD to T1, mix by pipetting.

25 Add 240 µL NWRB to T2, mix by pipetting.

26 Use T2 as a negative control for sorting.

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27 Sort nuclei from P3 gate into a 1.5 ml microfuge tube preloading 15 µL NWRB.

28 Collect 90,000 events from P3 gate, the final volume is about 40 µL - 60 µL.

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

30 Remove supernatant and resuspend pellet with 15 µL NWRB.

31 Use 8.4 µL of nuclei (50,400 events) for 10x GEM generation and barcoding following manufacture´s protocol.

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