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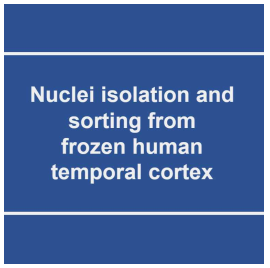
Nuclei Isolation and Sorting from Frozen Human Temporal Cortex_V2 V.2



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We use this protocol and it's working

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Abstract

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

Guidelines

This protocol need prior approval by the user's institutional review board (IRB) or equivalent ethics committee.

Materials

1. Dounce tissue grinder set **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D8938-1SET
2. Nuclei Isolation Kit: Nuclei PURE Prep **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**NUC201-1KT
3. DTT **Merck MilliporeSigma (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 **Takara Bio Inc. 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
10. Flowmi™ Cell Strainer 40 µm **Bel-Art Catalog #**H13680-0040
11. DAPI **Thermo Fisher Scientific Catalog #**D1306

Buffer Recipes:

1. Lysis buffer (LB):
- Nuclei PURE lysis Buffer
- 1:1000 DTT
- 0.01% Triton-x-100

2. Nuclei wash and resuspension buffer (NWRB):
- 1x DPBS
- 0.01% BSA
- freshly added RNase Inhibtior (40 U/ml)

3. Nuclei wash and resuspension buffer with DAPI (NWRBD):
- 1x NWRB
- 0.01 mg/ml DAPI

Safety warnings

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

Before start

Prepare Buffers as described in section '**Materials**'.

Method

15m

1 Cut at total of 10 sections of human temporal cortex (50 µm thickness) store on dry ice.

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

3 Add 2 mL LB to the tissue grinder.

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

5 Transfer the homogenate into a 2 ml microcentrifuge tube.

6 Incubate On ice for 00:05:00, mix with Wide bore tips once during the incubation. 5m

7 Filter the homogenate with a 70 µm strainer.

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

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

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

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

12 Remove the supernatant and add 1500 µL NWRB to the pellet without resuspension

13 Centrifuge at 500 x g, 4°C, 00:05:00 . 5m

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

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 NWRBD . and filter through 40µm FLowmi cell strainer (Bel-art)

18 Next, DAPI-stained nuclei were sorted using FACSARIA III cell sorter (BD Bioscience)

19 Gating strategy:
- use 100 µm nozzle
- FSC-A at 5V / SSC-A at 180V gating
- second gating is DAPI-postive nuclei using violet laser (405nm) with 450/50 bandpass filer
- Collect approximately 90,000 events in 1.5ml eppendorf tube and count on hemocytometer for quality control

20 Immediately after collection centrifuge at 500 x g, 4°C, 00:05:00 .

21 Remove supernatant and resuspend pellet according to the input amount in NWRB, For 90,000 events you resuspend in 15µl NWRB if you have less number of nuclei adjust the total volume accordingly.

22 Use 8.4 µL of nuclei (50,400 events) for 10x GEM generation and barcoding following manufacture´s protocol. Continue with GEM generation as per manufacture's instructions.

Protocol references

Habib et al 2017; pmid-28846088