Brain Cells Nuclei Isolation Multiome (10x Genomics PN 1000283, PN 1000285) (Mario Skarica / Nenad Sestan Lab) 20220525v3

INTRODUCTION:

The Brain Cell Nuclei Isolation is a comparable modification of our previous protocols (Zhu et al., 2018 Science; PMID: 30545855; Franjic et al. 2019 Biorxiv; doi: https://doi.org/10.1101/2019.12.31.889139) with yield improvement for limited tissue (adult and prenatal) and additional removal of debris. It has been additionally tested and adapted for Chromium Single Cell Multiome ATAC + Gene Expression (10x Genomics PN 1000283, PN 1000285)

Tissue preparation: To avoid experimental bias and evenly dissociate and randomize the tissue for cell nuclei isolation, whole tissue should be preferably finely pulverized to powder in liquid nitrogen with mortar and pestle (Coorstek #60316, #60317). However, with small regions that is not always possible, and one may lose tissue in liquid nitrogen pulverization.

Buffers: All buffers are ice cold and all reagents used for consequent nuclear isolation are molecular biology grade unless stated otherwise.

PROTOCOL STEPS:

1. Add ~20 mg of pulverized/whole tissue into 3 ml of ice-cold **Buffer A**.

2. Transfer the suspension was to Dounce tissue grinder (autoclaved, RNAse free, ice-cold) and homogenize **gently** with loose and tight pestles, 30 cycles each, with constant pressure and without introduction of air.

3. Strain the homogenate through 40 micrometer tube top cell strainer pre-wetted with **Buffer A** into polypropylene 50 ml tube.

4. Mix the final 3 ml of solution with 3 ml of **Buffer B** by washing the cell strainer with added solution.

5. Invert the tube 10x to mix well, and carefully pipette all 6 ml into one polypropylene 15ml centrifuge tube.

6. Centrifuge the suspension at 1000g, for 30 min at 4 ˚C on centrifuge (Eppendorf #5804R) and swing-out rotor (Eppendorf #S-4-72).

7. Remove the supernatant carefully and completely without touching the pellet and add total of 0.5 ml of **Buffer C** with P1000 tip and resuspend gently 10x with 400 microliter set-up on P1000, **without introduction of air**. Transfer all to 1.5 ml polypropylene tube. Leave on ice, undisturbed, for 5 minutes.

8. Add additional 0.5 ml of **Buffer D** and resuspend gently 10x with 900 microliter set-up on P1000, **without introduction of air.** Leave on ice, undisturbed, for 5 minutes.

9. Filter (pipette directly) through 35 micrometer tube top cell strainer pre-wetted with **Buffer D**.

10. Take 20 microliter sample **(Counts1)** for counts on hemocytometer.

11. Centrifuge the suspension at 500g, for 10 min at 4 ˚C on the same centrifuge and rotor.

12. Count the sample **(Counts1)** on the hemocytometer to find out the approximate expected yield.

13. Carefully and completely remove supernatant with pipettes in series: P1000, P200, P20 (last 10 microliters). Immediately add small volume of **Buffer E** based on expectation in **(Counts1)** and pipette gently 10x with P200 pipette tip, with set-up on P200 smaller than volume **to not introduce air**.

14. Filter (pipette directly) through 35 micrometer tube top cell strainer pre-wetted with **Buffer E** (remove all the extra buffer from filter) into the new 1.5 ml polypropylene tube.

15. Take minimal sample (2-5 microliters), dilute it 10x with **Buffer E** for **(Counts2)** on hemocytometer and adjust the nuclei concentration to 3.3 million per milliliter with **Buffer E**. Use the nuclei in **Chromium Next GEM Single Cell Multiome ATAC + Gene Expression** as soon as possible with recovery target of 10000 nuclei (use 5ul final nuclei solution).

SAVING EXTRA NUCLEI:

16. Add 1 ml of **Buffer D** and gently mix with leftover suspension of nuclei (use P1000).

17. Centrifuge the suspension at 500g, for 10 min at 4 ˚C on the centrifuge (Eppendorf #5804R) and rotor (Eppendorf #S-4-72).

18. Remove all the supernatant with pipette P1000, then P200 and add BamBanker media (GC Lymphotec #BB01) to the pellet. Gently resuspend the visible pellet with P1000, **do not introduce air**, and transfer to a pre-chilled 2 ml Nunc cryovial. Adjust the concentration to approximately 1 million per milliliter.

19. Slow-freeze nuclei in a 4°C preconditioned freezing container (Corning® CoolCell™ LX Cell Freezing Container) at -80°C and move to permanent storage at -80°C after equilibration, in approximately 90 minutes.

USING EXTRA NUCLEI:

20. Remove nuclei from -80°C and leave them on ice to thaw fully.

21. Resuspend them gently with P1000 and take the needed volume and add 1 ml of **Buffer D**, gently mix them.

22. Centrifuge the suspension at 500g, for 10 min at 4 ˚C on the on the centrifuge (Eppendorf #5804R) and rotor (Eppendorf #S-4-72).

23. Repeat steps 7. to 15. - additionally, if you observe clumping of nuclei, it is beneficial to filter them through 20 micrometer strainer (Pluriselect #43-10020-50), by pipetting directly through it with P200 to minimize loss of volume.

BUFFERS:

CRITICAL: *DTT, RNAse Protector, Protease Inhibitors - add when preparing buffers* ***before immediate use****. Igepal CA-630 - add* ***just before start*** *of Dounce tissue homogenization*

Buffer A (in Nuclease Free Water):
250 mM sucrose (Sigma #S0389)
25 mM KCl (Sigma #60142)
5mM MgCl2 (Sigma #M1028)
20mM Tris-HCl (pH 7.5) (AmericanBio #AB14043; Sigma #T2413)
Protease Inhibitors w/o EDTA (1 tablet/10ml buffer) (Roche #11836170001)
RNAse inhibitor (80U/ml) (Roche #03335402001)
1mM DTT (Sigma #43186)
0.1% (v/v) Igepal CA-630 (Sigma#I8896)

Buffer B (in Nuclease Free Water):
50% iodixanol (v/v), (Optiprep, Axis-Shield #1114542; **60% Iodixanol concentrate**),
25 mM KCl (Sigma #60142),
5mM MgCl2 (Sigma #M1028),
20mM Tris-HCl (pH 7.5) (AmericanBio #AB14043; Sigma #T2413),
Protease Inhibitors w/o EDTA (1 tablet/10ml buffer) (Roche #11836170001),
RNAse inhibitor (80U/ml) (Roche #03335402001),
1mM DTT (Sigma #43186)
1% (m/v) BSA (Gemini Bio-Products #700-106P)

Buffer C (in Nuclease Free Water):
10mM Tris-HCl (pH 7.4) (AmericanBio #AB14043; Sigma #T2413)
10mM NaCl (Sigma #71386)
3mM MgCl2 (Sigma #M1028)
1mM DTT (Sigma #43186)
RNAse inhibitor (1000U/ml) (Roche #03335402001)
0.01% (v/v) NP-40 (Sigma #74385)
0.01% (v/v) TWEEN-20 (Bio-Rad #166-2404)
0.001% (v/v) Digitonin (Thermo Fisher #BN2006)
1% (m/v) BSA (Gemini Bio-Products #700-106P)

Buffer D (in Nuclease Free Water):
10mM Tris-HCl (pH 7.4) (AmericanBio #AB14043; Sigma #T2413)
10mM NaCl (Sigma #71386)
3mM MgCl2 (Sigma #M1028)
1mM DTT (Sigma #43186)
RNAse inhibitor (1000U/ml) (Roche #03335402001)
0.1% (v/v) TWEEN-20 (Bio-Rad #166-2404)
1% (m/v) BSA (Gemini Bio-Products #700-106P)

Buffer E (in Nuclease Free Water):
1X Nuclei Buffer (from 20X) (10x Genomics #2000153/2000207)
1mM DTT (Sigma #43186)
RNAse inhibitor (1000U/ml) (Roche #03335402001)

Equipment, Materials and Reagents List:

BSA (Gemini Bio-Products #700-106P)
KCl (Sigma #60142)
MgCl2 (Sigma #M1028)
NaCl (Sigma #71386)
Sucrose (Sigma #S0389)
Optiprep (Axis-Shield #1114542; 60% Iodixanol concentrate)
Tris-HCl (pH7.4; pH 7.5) (AmericanBio #AB14043; Sigma #T2413)
Water, Nuclease-Free, Non-DEPC Treated (American-Bio #AB02123)
DTT (Sigma #43186)
Protease Inhibitors w/o EDTA (Roche #11836170001)
RNAse inhibitor (Roche #03335402001)
Digitonin (Thermo Fisher #BN2006)
NP-40 (Sigma #74385)
Igepal CA-630 (Sigma#I8896)
Tween 20 (Bio-Rad #166-2404)
Mortar and Pestle (Coorstek #60316, #60317)
Dounce Tissue Grinder (Wheaton 15ml; #357544)
40 micrometer tube top cell strainer (Corning #352340)
35 micrometer tube top cell strainer (Corning #352235)
1.5 ml tube (Corning Axygen #MCT-150-L-C)
15 ml tube (Corning #352099)
50 ml tube (Corning #430290)
Centrifuge (Eppendorf #5804R)
Rotor (Eppendorf #S-4-72)
Corning® CoolCell™ LX Cell Freezing Container (Corning#CLS432001)
Strainer; 20 micrometer (Pluriselect #43-10020-50)
Media, BamBanker (GC Lymphotec #BB01)