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Human Fixed Nucleus Isolation for Single-Nucleus Transcriptomic Profiling (10x Genomics)

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol for generating suspensions of fixed human nuclei for single-nucleus transcriptomics.

Equipment and Reagents

1 **Equipment**

- Kimble Dounce Kontes tissue-grinder set (DWK 885300-0000)
- 50 ml Oakridge tubes (#0556214D) // can replace with 50 mL Falcon Tubes
- 15 mL Falcon tubes (Fisher #352097)
- 50 mL Falcon tubes (Fisher #352070)
- 1.5mL LoBind Eppendorf Tubes
- 70-micron Corning Cell Strainer (#431751)
- Fire polished glass Pasteur pipettes (VWR #14672-380, polished in an open gas flame down to ~600 micron, 300 micron and 150 micron tip opening sizes) // alternatively can replace with regular pipetting

2 **Reagents**

- Roche Protector RNase Inhibitor (Millipore Sigma RNAINH-RO)
- 1M DTT (dithiothreitol, prepare fresh every couple of months and store at -20°C)
- Ultrapure RNA-se free/ DNA-se free water

3 **Protocol Outline**

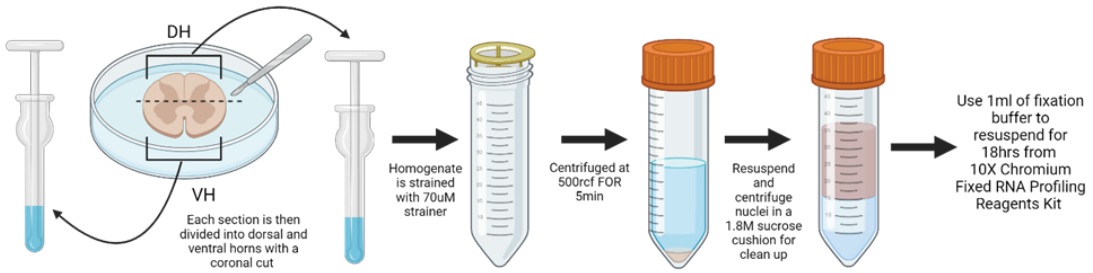

Figure 1: Protocol outline with spinal cord as sample central nervous system tissue.

Solutions

4 **NMDG-Hepes-ACSF**

- NMDG (93 mM)
- KCl (2.5 mM)
- NaH_2PO_4 (1.2 mM)
- $NaHCO_3$ (30 mM)
- HEPES (20 mM)
- Glucose (25 mM)

Bring pH to between 7.3 - 7.4 with 10N HCl and filter sterilize (good for 2 weeks at 4°C).

On the morning of tissue preparation add the following components (final concentration):

- Na-Ascorbate (5 mM)
- Thiourea (2 mM)
- Na-pyruvate (3 mM)

- $MgSO_4$ (10 mM, prepare 2M stock that is good for 6-months at 4°C)
- $CaCl_2$ (1 mM, prepare 2M stock that is good for 6-months at 4°C)
- Kynurenic acid Na-salt (1 mM)

5 **Nuclear Buffer**

- Sucrose (320 mM)
- Tris-HCl (pH=7.4) (10 mM)
- $MgCl_2$ (3 mM)
- NaCl (10 mM)
- BSA (RNAse free) (0.50%)
- Kollidon VA64 (1 %)
- Ultrapure water, fill to 50 mL and 0.22 micron filter sterilize.

Morning of run:

- DTT (dithiothreitol, 1 mM)
- Roche Protector RNAse Inhibitor (0.1 U/uL)

6 **Lysis Buffer**

- Nuclear buffer
- Triton-X100 (0.1%)

7 **1.8M Sucrose Cushion**

Sucrose (1.8 M)
Tris-HCl (pH=7.4) (10 mM)
$MgCl_2$ (3 mM)
NaCl (10 mM)
BSA (nuclease free) (0.50%)
Kollidon VA64 (1%)

Water (ultrapure) Fill to 50 mL
Do NOT Filter sterilize!

Protocol

8 **1. Prepare solutions and equipment**

- Prepare 50 mL of NMDG-HEPES-ACSF from pre-prepared stock by adding (Na-Ascorbate, Thiourea, Na-pyruvate, $MgSO_4$, $CaCl_2$ and Kynurenic acid Na-salt) and place on ice.
- Prepare Nuclear Buffer (add DTT and RNA-se inhibitor to preprepared solution) and place on ice.
- Prepare Lysis Buffer from Nuclear Buffer (add Triton-X100 to 0.1% of final volume) and pipette 0.75 mL into a Kontes tissue grinder.
- Prepare 1M sucrose cushion (add DTT and RNA-se inhibitor to preprepared solution) and place on ice.
- Pre-cool centrifuge to 4°C.
- Place 100 mm dissection dish into a 150 mm dish with dry ice.

9 **2. Dissect out tissue**

Place snap frozen brain tissue into 100 mm tissue culture dissection dish on a layer of dry ice in a larger 150 mm dish. Microdissect out desired tissue parts and cut into small 1.5 mm^3 cubicles. Drop the latter into ice-cold NMDG-Hepes-ACSF in 1.5 mL collection tubes on ice.

10 **3. Generate nuclear suspension**

- Transfer tissue pieces into the Lysis Buffer in the Kontes tissue grinder.
- Apply 5 strokes with the loose pestle followed by 15 strokes with the tight pestle.
- Place a 70-micron cell strainer on a 50 mL Falcon tube and pre-wet with 500 µL of Nuclear Buffer.
- Add 250 µL of Nuclear Buffer to the tissue grinder.
- Mix nuclear suspension in tissue grinder twice with a 600-micron fire polished glass capillary and transfer through the cell strainer.
- Wash tissue grinder with 750 µL Nuclear Buffer and transfer again through the cell strainer.
- Wash cell strainer with final 750 µL Nuclear Buffer.

11 **4. Spin nuclei down and resuspend in fresh Nuclear Buffer**

- Spin nuclei down for 5 min at 500g at 4°C in a spin-out rotor.
- Remove supernatant and resuspend in fresh 3 mL Nuclear Buffer.

12 **5. Purify nuclei with a sucrose cushion centrifugation**

- Transfer 12 mL of Sucrose Cushion into a 50 mL Oakridge tube.
- Gently layer the nuclear suspension from the previous step on the sucrose cushion (avoid mixing of the layers).
- Centrifuge the tubes at 3200g at 4°C for 20 minutes.
- After centrifugation, pour out the supernatant by decanting in one smooth motion and drying out the neck of the Oakridge tube with a Kimwipe.
- Resuspend the nuclear pellet in 100 µL of ice-cold Nuclear Buffer and mix gently with a 300 micron fire polished Pasteur pipette.
- Transfer purified nuclear suspension to a new 15 mL tube on ice.

13 **6. Evaluate debris**

- Pipette 3 µL of the nuclear suspension on a glass slide and evaluate debris under a brightfield microscope

- If there is a lot of debris, add 2ml of Nuclear Buffer to the nuclear suspension and spin nuclei down at
500g for 5 min at 4C in a spin-out-rotor.

14 **7. Fix nuclei and proceed with profiling**

- Remove supernatant and resuspend nuclei in 1mL of Fixation buffer from the 10X fixation of cells & nuclei for Chromium fixed RNA profiling (CG000478) and transfer resuspension to a microcentrifuge tube and incubate for 18hr at 4°C.
- Continue with 10x fixation protocol.