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Nuclei Isolation

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

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Abstract

nuclei isolation for snRNA-seq

1 Prepare buffers and filter sterilize, add RNAse inhibitor fresh NP40 Lysis Buffer (NST): 0.1% NP-40 Alternative (or NP-40), [M] 10 millimolar (mM) Tris, [M] 146 millimolar (mM) NaCl, [M] 1 millimolar (mM) CaCl2, 21mM MgCl2, 40U/mL of Protector RNAse inhibitor (add fresh day of) ST Wash Buffer: (10mM Tris, [M] 146 Mass Percent NaCl, [M] 1 millimolar (mM) CaCl2, 21mM MgCl2), 0.01% BSA, 40U/mL of Protector RNAse inhibitor (add fresh day of) ST Staining buffer (ST-SB): 2%BSA, 0.02%Tween-20, [M] 10 millimolar (mM) Tris, [M] 146 millimolar (mM) NaCl, 1mM CaCl2, 21mM MgCl2), 40U/mL of Protector RNAse inhibitor (add fresh day of) Note: Keep tissues/homogenate and buffers on ice throughout the protocol. Pre-cool the centrifuge to 4 °C and keep at 4 °C for all steps.

2 Tissue collection a) Sacrifice and rapidly decapitate mice. Using chilled brain matrix (Ted Pella), cut a thick coronal section from 5mm back from start of cortex until start of cerebellum. Store section in ice-cold Hibernate-A media in 10cm plate on ice until all brains have been dissected. b) Use a razor blade to dissect out midbrain and collect tissue, placing directly into dounce homogenizer.

3 Tissue lysis and homogenizing a) For each sample to barcode and pool: prepare a separate homogenizer and douncing pestles (loose and tight). Add 1 mL NST buffer to the tube with tissue and transfer to dounce homogenizer and keep on ice. b) with a total volume of 1 mL, dounce 20 times with the loose pestle followed by 20 times with the tight pestle. c) Add 1 mL of ST wash buffer, filter through 30µm filters (Milentyi Biotec 130-041-407) and transfer filtered homogenate to a 15mL tube. d) Rinse the homogenizer with 3x 1 mL of ST wash buffer, filter through 30µm filters and add to the filtered homogenate to add up to a final volume of 5 mL. e) Immediately spin down at 500 g for 00:05:00 at 4 °C to pellet the nuclei in swing bucket rotor f) Remove supernatant g) Resuspend nuclei in 200µl of ST-SB and transfer to lo-bind 1.5 mL tube and wash out the original tube with an additional 1 mL and transfer to the same 1.5ml tube. h) Wash by spinning down for 00:05:00 at 500 g at 4 °C and resuspending in 1.2 mL ST-SB twice, for a total of 3 washes i) resuspend in 1 mL ST-SB

10m

4 Proceed with sorting nuclei for GFP+ nuclei via MACS Tyto