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Single Nucleus RNAseq Sample Prep from Nodose Ganglia

Sebastian Preissl[1], Jamie Verheyden[1], Xin Sun[2]

[1]University of California, San Diego; [2]University of California-San Diego

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How to isolate single nucei from nodose vagal ganglia neurons in preparation for 10XGenomics sequencing.

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MATERIALS

liquid nitrogen **Contributed by users**

DTT **Sigma**

Aldrich Catalog #D0632

BSA **Sigma**

Aldrich Catalog #A7906

RNasin **Promega**

PBS **Contributed by users**

Trypan Blue 100 mL **Stemcell**

Technologies Catalog #7050

cOmplete™, Mini Protease Inhibitor

Cocktail **Roche Catalog #11836153001**

EDTA **Invitrogen - Thermo**

Fisher Catalog #AM9261

DRAQ7 **BD**

Biosciences Catalog #564904

Lysis buffer (LB):-> 550ul per sample

Prepare fresh and put on ice

Reagent	Stock concentration	Final concentration	1 sample	2 samples
TritonX-100	10%	0.2%	11 ul	22 ul
Roche protease inhibitor	25x	1x	22 ul	44 ul
DTT	200mM	1mM	2.75 ul	5.5 ul
RNAsin (Promega, N211B)	40 U/ul	0.2U/ul	2.75 ul	5.5 ul
2 % BSA in PBS			512.5 ul	1025 ul

Sort buffer (SB): -> 1ml per sample

Prepare fresh and put on ice

Reagent	Stock concentration	Final concentration	1 sample	5 samples
EDTA (Life technologies)	500mM	1mM	2 ul	10 ul
RNAsin (Promega, N211B)	40 U/ul	0.2U/ul	5 ul	25 ul
2 % BSA in PBS			993 ul	4.97 ml

Collection buffer (CB): -> 200ul per sample

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Prepare fresh and put on ice

Reagent	Stock concentration	Final concentration	1 sample	5 samples
RNAsin (Promega, N211B)	40 U/ul	1U/ul	5 ul	25 ul
5 % BSA in PBS			195 ul	975 ul

Reaction buffer (RB): -> 20ul per sample

Prepare fresh and put on ice

Reagent	Stock concentration	Final concentration	1 sample	5 samples	10 samples
5% BSA (Sigma, Molec boil grade, B6917)	5%	1%	4 ul	20 ul	40 ul
RNAsin (Promega, N211B)	40 U/ul	0.2U/ul	0.25 ul	1.25 ul	2.5 ul
PBS			15.75 ul	78.75 ul	157.5 ul

1 Nodose ganglia are dissected and flash frozen in liquid nitrogen.Store at -80C until ready to isolate nuclei.Grind up the sample with liquid nitrogen using the 1.5mL centrifuge tube and fitting pestle.

2 Add 500 ul Lysis Buffer to pulverized tissue, pipette 10x up and down and place on ice Incubate 5 min with the overhead shaker in the cold room

3 Spin down 5 min 500g at 4C using soft settings. Remove supernatant

4 Resuspend tissue in 400 ul Sorting Buffer by pipetting 10x up and down.

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5 Filter with a green 30 um CellTRic (Sysmex) into FACS tube. Wash filter with another 200 ul SB. Gently clap rack against bench to fully transfer the nuclei suspension. Add 6 ul DRAQ7 (Cell signaling) to stain nuclei and place sample on ice.

6 For collection of sorted nuclei add 50 ul Collection Buffer

to a 1.5 ml LoBind tube (collection tube).

7 Use 100 um Chip for sorting of nuclei
Set temperature for sample and collection to 5C
Identify single nuclei based FSC/SSC signals andDRAQ7 fluorescence in FL-6 (PE-Cy7) channel

8 Sort 60,000 (200 ul) nuclei (Gate: “Sorting”) with the setting “Purity” into the prepared collection tube. After sorting is finished (10-20 min), mix sample and keep on ice.

9 Spin down 15 min 1000xg at 4C (Using swinging bucket rotor). After centrifugation, there should be a tiny blue pellet be visible. Remove supernatant and resuspend in 18 ul RB by pipetting 10x (Recovery of nuclei should be around 50-60%). Leave sample on ice.

10

Check for single cell suspension and count nuclei using hemocytometer. Mix 5 ul sample + 5 ul Trypan blue and count 4 big squares.
Calculation: (counted number)/4*20=nuclei/ul). Adjust concentration to 1000 nuclei/ul. Follow 10xGenomics library prep protocol.

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