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Single nuclear isolation

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We use this protocol and it's working

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Abstract

Single nuclear isolation protocol adapted from Nadelmann, et. al. doi: **10.1002/cpz1.132**. This protocol has been tested on mouse liver tissue stored in RNALater and flash frozen in liquid nitrogen then stored in -80C freezer.

Reagent Preparation

1 Nuclei isolation buffer 2 (NIM2)
For 1 sample,

- NIM1 (see above) - 1246.25 ul
- 1 mM DTT (Fisher Scientific PI20290) - 1.25 ul - 1uM
- 50x Protease Inhibitor (Sigma 11873580001) - 25 ul

total volume: 1250 ul

2 Nuclei isolation buffer 1 (NIM1)
For 1 sample,

- 1.5 M sucrose (Sigma @0389) - 625 ul - 250mM
- 2 M KCl (Ambion AM9640G) - 46.875 ul - 25mM
- 1 M $MgCl_2$ (Ambion AM9530G) - 18.75 ul - 5 mM
- 1 M TrisCl, pH 8 (Ambion AM9855G) - 37.5 ul - 10 mM
- Nuclease-free water - 3021.88 ul

total volume: 3750 ul

3 Homogenization buffer (HB)
For 1 sample,

- NIM2 (see above) - 1212.5 ul
- 40 U/ul RNaseIn (Ambion AM2682) - 12.5 ul - 0.4 U/ul
- 20 U/ul SuperaseIn (Ambion AM2694) - 12.5 ul - 0.2 U/ul
- 10% (v/v) Triton X-100 (Sigma T8787) - 12.5 ul - 0.10%

total volume: 1250 ul

4 Storage buffer (SB)
For 1 sample,

- Phosphate-buffered saline (Leinco Technologies P349) - 995ul
- Bovine serum albumin (Sigma A9647) - 40mg - 4%
- 40 U/ul Protector RNaseIn (Sigma 03 335 402 001)- 5 ul - 0.2 U/ul

total volume: 1000ul

Sample Preparation

5 At harvest, store samples in RNALater (Invitrogen AM7021) and flash freeze in liquid nitrogen. Store in -80C freezer.

Nuclei Isolation 10m

6 Thaw RNALater stored tissue on ice.

Nuclei Isolation 10m

7 Remove tissue with transfer pipette and place into a wide-based 2ml Eppendorf tube with 1000ml of homogenization buffer. (use sharp dissection to break tissue into smaller chunks)

8 Place 2 large (3mm) and 3 small (2.3mm) homogenization beads

9 Set temperature of tissue homogenizer (Servicebio SWE-3D) to -20C. Homogenize tissue at 35Hz for 60s. (Difficult tissue may be run up to 60Hz for 60s; do not run more than twice).

10 All samples to lyse on ice for 5 minutes 5m

11 Filter homogenized tissue through 40-um cell strainer into a non-stick RNAase-free 1.5ml tube

12 Centrifuge 500g for 5 minutes at 4C 5m

13 transfer the supernatant into new 1.5ml RNAase-free tubes (can be used for bulk RNA sequencing)

14 Resuspend nuclei pellet with 500ul of SB

15 Filter 250ul into FACS tubes

16 Spin FACS tube down at 200g for 20s at 4C

17 Choose a negative control (typically a sham); separate 100ul of sample into a new FACS tube

18 Add 2 drops of nucblue in all FACS tubes but control

19 Vortex 5s and let the samples sit for 15 minutes

Flow Cytometry

20 Run the negative control to set up the gate

21 Gating strategy: SSC-A vs FSC-A → SSC-H vs SSC-W → FSC-H vs FSC-W → Hoechst-A vs SSC-A (doublet strands are typically seen)

22 Sort the entire sample to maximize yield (approximately 30 minutes) into 1.5ml non-stick RNase-free micro centrifuge tubes. Keep on ice until all sorting is completed.

Counting Nuclei

5m

23 Centrifuge samples 500g for 5min at 4C

5m

24 Remove supernatant carefully (save supernatant)

25 Re-suspend nuclei pellet according to FACS Cell numbers
- <50,000 - 30 ul
- 50,000-100,000 - 50ul
- 100,000-150,000 - 70 ul
- 150,000+ - 90ul

26 Count nuclei using Countess. Add 6ul of sample with 6ul of trypan blue then load 10ul of solution into Countess chamber. OPTIONAL use automatic cell concentration device. (ideal concentration is 8×10^5 - 1.2×10^6)

Protocol references

Nadelmann, E. R., Gorham, J. M., Reichart, D., Delaughter, D. M., Wakimoto, H., Lindberg, E. L., Litviňukova, M., Maatz, H., Curran, J. J., Ischiu Gutierrez, D., Hübner, N., Seidman, C. E., & Seidman, J. G. (2021). Isolation of nuclei from mammalian cells and tissues for single-nucleus molecular profiling. *Current Protocols*, 1, e132. doi: **10.1002/cpz1.132**