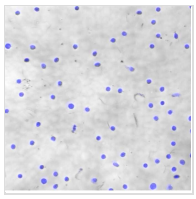

Oct 21, 2022

Nuclei extraction for single-cell RNAseq from frozen tissue using Singulator™ 100

Forked from Nuclei extraction for single-cell RNAseq from frozen tissue using Singulator™ 100 (Citric Acid preservation)

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1 *Works for me* Share

This protocol is published without a DOI.

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ABSTRACT

This protocol describes nuclei extraction from snap frozen tissue (human or mouse) using Singulator, including washing nuclei suspension in buffer supplemented with sucrose, and nuclei FACS sorting. The resulting nuclei suspension is suitable for scRNA-seq using a platform of choice (as 10X Chromium, or inDrops). The protocol has been validated on various snap frozen human and mouse tissues (lung, brain, prostate, tumors, core needle biopsies amongst others).

PROTOCOL CITATION

Ignas Masilionis, Ojasvi Chaudhary, Ronan Chaligne, Linas Mazutis 2022. Nuclei extraction for single-cell RNAseq from frozen tissue using Singulator™ 100. **protocols.io** https://protocols.io/view/nuclei-extraction-for-single-cell-rnaseq-from-froz-b7vxrn7n

FORK NOTE

FORK FROM

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KEYWORDS

Nucseq, RNAseq, Single Cell, Singulator, Nuclei

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MATERIALS TEXT

MATERIALS

DTT **Sigma Aldrich Catalog #D0632**

BSA **Sigma Aldrich Catalog #A7906**

4,6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI) **Thermo Fisher Scientific Catalog #D1306**

Protector RNase Inhibitor **Sigma – Aldrich Catalog #3335402001** Step 3

Nuclease-Free Water (not DEPC-Treated) **Thermo Fisher Scientific Catalog #AM9937** Step 3

7-AAD (7-Aminoactinomycin D) **Thermo Fisher Catalog #A1310**

Preparation 10m

1 10m

- Turn on and pre-cool Singulator **00:10:00**

- Make sure there is sufficient number of Nuclei cartridges stored at **4 °C**

2 10m

Take the tissue out of LN2 or -80C freezer and place it on dry ice to keep it frozen
Piece should be <100mg, if not cut it in smaller pieces (<100mg and >10mg).

Cutting can be done with razor blade and within a petri dish on dry ice. Hold the tissue with cooled and RNAse free tweezers (be careful it might fly off). Work fast to not let the tissue thaw while cutting. Place tissue back in a tube on dry ice immediately after cutting.

3 5m

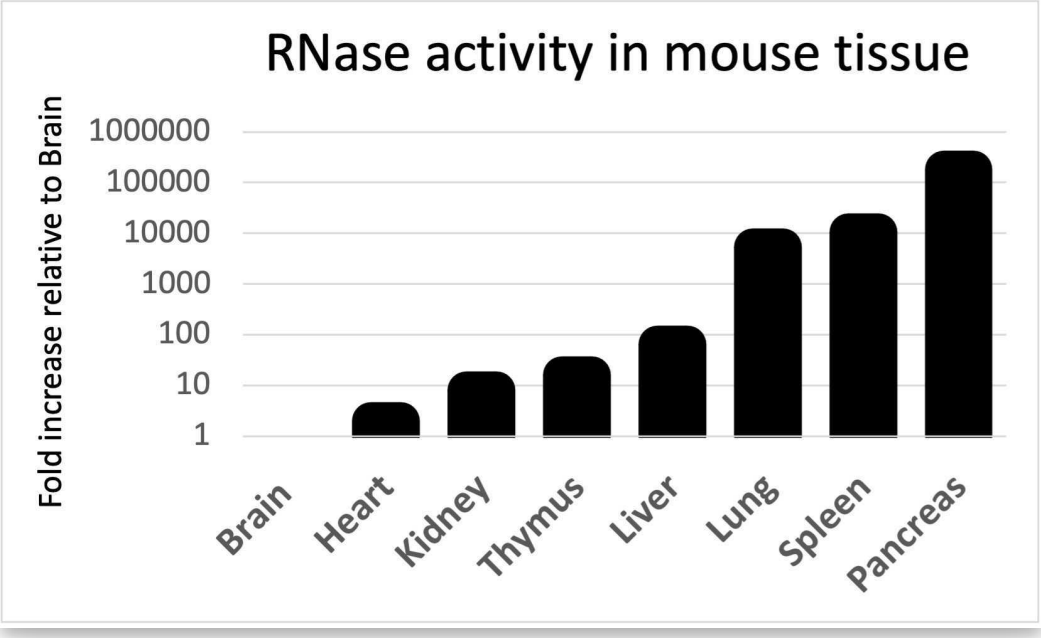
Prepare 2mL of Nuclei Wash Buffer per sample.

1X PBS final
BSA: 1% final
DTT: [M]**1 millimolar (mM)**
0.2 - 1.0 U/ul RNAse inhibitor (depending on tissue type, see RNase Activity in Mouse Tissue below) **(**

☒Protector RNase Inhibitor **Sigma – Aldrich Catalog #3335402001**)

Please be sure to use Nuclease-free water (i.e. ☒Nuclease-Free Water (not DEPC-Treated) **Thermo Fisher Scientific Catalog #AM9937**)

https://www.thermofisher.com/us/en/home/references/ambion-tech-support/nuclease-enzymes/tech-notes/rnase-activity-in-mouse-tissue.html



Singulator 10m

4 Add RNAse inhibitor (0.2-1.0 U/uL final depending on tissue type - Final volume ~ 3.5 mL for standard protocol, ~2.5 for low volume) and DTT (1M, final 1mM) directly into Nuclei Isolation Cartridge just before the run. 10m

Transfer tissue into the cooled Nuclei Isolation Cartridge and start Nuclei isolation protocol.

Post-singulator nuclei washes 10m

5 When run is over on Singulator, transfer nuclei suspension (~3.5mL for standard protocol) into two 2 mL Protein LoBind Tubes (Eppendorf, 0030108450) (split evenly, ~1750 uL each). 5m
1.5mL Protein LoBind Tubes (Eppendorf) can be used for low yield samples i.e. biopsies and/or low volume protocol.

6 Add 250uL of [M]**2 Molarity (M)** Sucrose solution to both tubes ([M]**250 millimolar (mM)** final). Invert the tube slowly 10 times to mix sucrose and nuclei suspension.

7 **500 x g, 4°C, 00:05:00 , Swinging bucket centrifuge** 5m

Washes and preparation for FACS 40m

8 After centrifugation, a layer of debris might be visible at the top and a pellet of nuclei at the bottom (for lower yields it might not be noticeable). 2m
Aspirate supernatant with a P1000 pipette, be really careful when removing the debris layer on the meniscus without disrupting nuclei pellet at the bottom of the tube (some liquid can be left to prevent pellet aspiration ~50uL).

9 Resuspend Nuclei pellet in 250uL Nuclei Wash Buffer, then using same pipette tip transfer this nuclei suspension to other tubes pooling all tubes together.

9.1 OPTIONAL: 5m

If there is still debris present, an additional 250mM sucrose wash can be performed:
Measure volume of nuclei suspension.
Add 2M sucrose to final concentration of 250mM, mix by inverting gently few times
500 x g, 4°C, 00:05:00 , Swinging bucket centrifuge

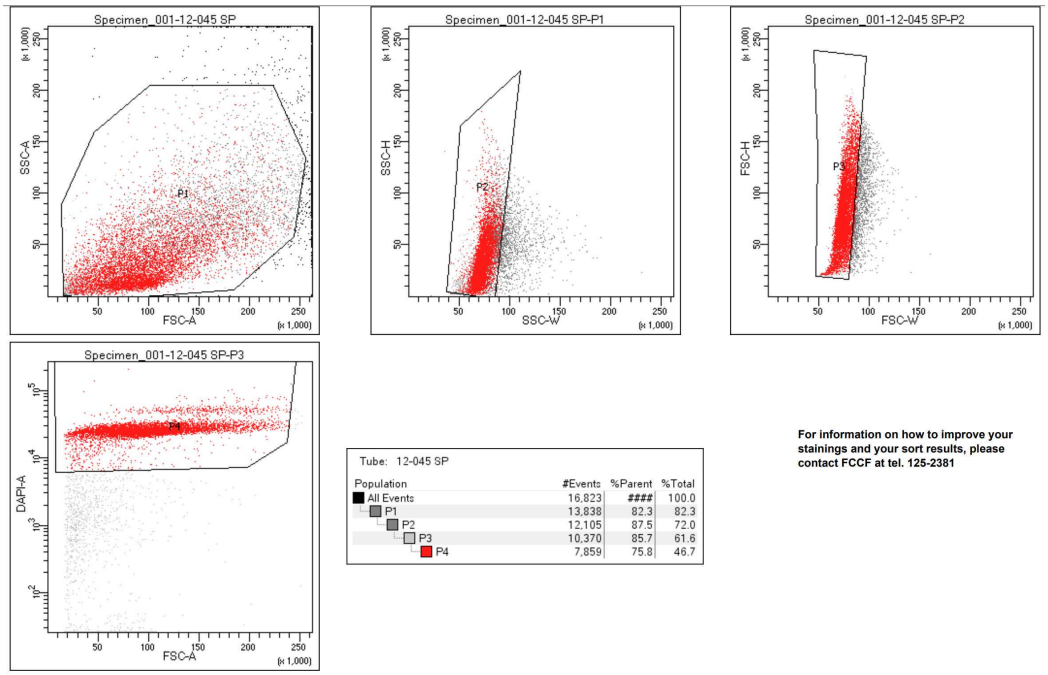
10 Remove supernatant and re-suspend in 250-500ul Nuclei Wash Buffer depending on expected yield. 2m
Filter nuclei suspension through a 35 µm FACS tube cap filter (blue) or FlowMi 40µm Filter.

11 Count nuclei using DAPI (or another nuclear stain) on Countess II cell counter. 5m
Trypan can also be used to count in Bright field.

(DAPI counts are more precise though)

12 **(OPTIONAL)** 30m
7-AAD positive nuclei (1 ug/mL final conc.) can be FACS sorted into 500uL of Nuclei wash buffer using 1.5mL Lobind Eppendorf as collection tube (see attached PDF for gating setup). Suggested nozzle size: 100um.

Sort_Report_DAPI_pos_Nuclei.pdf



After FACS wash and preparation for single nuclei encapsulation 10m

13 Concentrate sorted nuclei to ~1000 nuclei/uL and Count using DAPI on Countess II cell counter. 10m
Trypan can also be used to count in Bright field.
There should be no clumps, debris or nuclei aggregate if observed additional filtration can be applied through FlowMi 40µm tip Filter.

Encapsulation

14 Follow 10x protocol (scRNAseq 5' or 3' version) for encapsulation.
During cDNA amplification, increase cycle number to 14 when targeting 5000 nuclei.