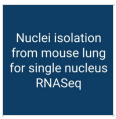

Apr 07, 2020

Nuclei isolation from mouse lung for single nucleus RNASeq

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1 Works for me dx.doi.org/10.17504/protocols.io.bdv2i68e

Jeffrey Koenitzer

ABSTRACT

This protocol is for **nuclei isolation from mouse lung for single nucleus RNASeq**.

It is adapted directly from Joshi et al., with adjustments to RNase inhibitor concentrations and removal of FACS sorting steps.

Nikita Joshi, Alexander Misharin. Single-nucleus isolation from frozen human lung tissue for single-nucleus RNA-seq. http://dx.doi.org/10.17504/protocols.io.zu8f6zw

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Koenitzer, J. R., Wu, H., Atkinson, J. J., Brody, S. L., Humphreys, B. D. (2020). Single nucleus RNASeq profiling of mouse lung: reduced dissociation bias and improved detection of rare cell types compared with single cell RNASeq. bioRxiv. preprint doi: https://doi.org/10.1101/2020.03.06.981407

ATTACHMENTS

Protocol for nuclei isolation from mouse lung for single nucleus RNASeq.pdf

step-by-step_Lung nuclear isolation protocol v1.pdf

MATERIALS

NAME	CATALOG #	VENDOR
RNaseZap®	AM9780	Thermo Scientific
cOmplete ULTRA Tablets, Mini, EDTA-free, EASYpack	05 892 791 001	Roche
RNasin Plus Ribonuclease Inhibitors	N2615	Promega
SUPERaseIN RNase Inhibitor	AM2696	Thermo Fisher Scientific
RNase free H2O	AM9938	Thermo Scientific
Albumin, Bovine Serum, 10% Aqueous Solution, Nuclease-Free	126615-25ML	Millipore Sigma
Gibco™ DPBS no calcium no magnesium	14190144	Thermo Fisher Scientific

Citation: Jeffrey Koenitzer, Ben Humphreys (04/07/2020). Nuclei isolation from mouse lung for single nucleus RNASeq. https://dx.doi.org/10.17504/protocols.io.bdv2i68e

NAME	CATALOG #	VENDOR
Nuclei Isolation Kit: Nuclei EZ Prep	NUC-101	Sigma Aldrich

MATERIALS TEXT

The *Nuclei Isolation Kit* contains the *Nuclei EZ Lysis Buffer* that is required for the praparation of Lysis Buffer and cOmplete stock (10x).

Storage Conditions

Material	Storage
1x DPBS	4 ºC
Nuclei EZ Lysis Buffer (from kit NUC-101)	4 ºC
cOmplete ULTRA Tablets, Mini, EDTA-free, EASYpack	4 ºC
RNasin Plus Ribonuclease Inhibitors	-20 °C
SUPERaseIN RNase Inhibitor	-20 °C
RNase free H2O	RT
RNaseZap	RT
Bovine serum albumin, 10% solution, nuclease free	-20 °C

gentleMACS™ C Tubes
Tissue dissociators and tubes
gentleMACS™ 130-093-237

gentleMACS™ Dissociator
Tissue Dissociator
MACS 130-093-235

pluriStrainer® 40 µm
Cell Strainer
pluriSelect 43-50040

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pluriStrainer® 5 µm
Cell Strainer
pluriSelect 43-50005

C-Chip™ Disposable Hemacytometers (Fuchs Rosenthal)
Counting Chamber
INCYTO DHCF015

Falcon™ 15mL Polystyrene Conical Centrifuge Tubes
Centrifuge Tubes
Falcon™ 352095

Ambion® RNase-free 50 ml Conical Tubes
Centrifuge Tubes
Ambion® AM12502

TPP 60 mm Tissue Culture Dishes
Tissue Culture Dish
TPP TP93060

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

BEFORE STARTING

Prepare Working Solutions

Complete stock (10x)

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- **1 ml Nuclei EZ Prep lysis buffer**
- **1 tablet cOmplete ULTRA tablets**

Lysis Buffer - 2 ml per < 6 mm^3 tissue

- **200 µl cOmplete stock**
- **1.775 ml Nuclei EZ Prep lysis buffer**
- **12.5 µl RNasin Plus**
- **12.5 µl SUPERaseIN**

Wash Buffer - 4 ml per < 6 mm^3 tissue

- **3.575 ml dPBS**
- **400 µl 10% BSA**
- **12.5 µl RNasin Plus**
- **12.5 µl SUPERaseIN**

Resuspension Buffer – 1 ml

- **0.977 ml dPBS**
- **10 µl 10% BSA**
- **6.25 µl RNasin Plus**
- **6.25 µl SUPERaseIN**

1 Pre-cool all instruments (including centrifuges), buffers, and tubes.

Note: All steps are performed **On ice** **or in cold room** (**4 °C**) to minimize RNA degradation.

2 Remove mouse lung sample from -80 ºC freezer and trim a **~6 mm^3 piece**.

3 Thaw on small plastic weighing boat until able to insert 26G needle to tissue, then inject ice cold **1 ml Lysis Buffer** to 'inflate' the tissue. Add the remaining Lysis Buffer and chop to the smallest pieces possible with scissors (60 s).

4 Transfer the minced tissue and buffer to a GentleMacs C tube.

5 Close, invert, and transfer directly to MACS Tissue dissociator.

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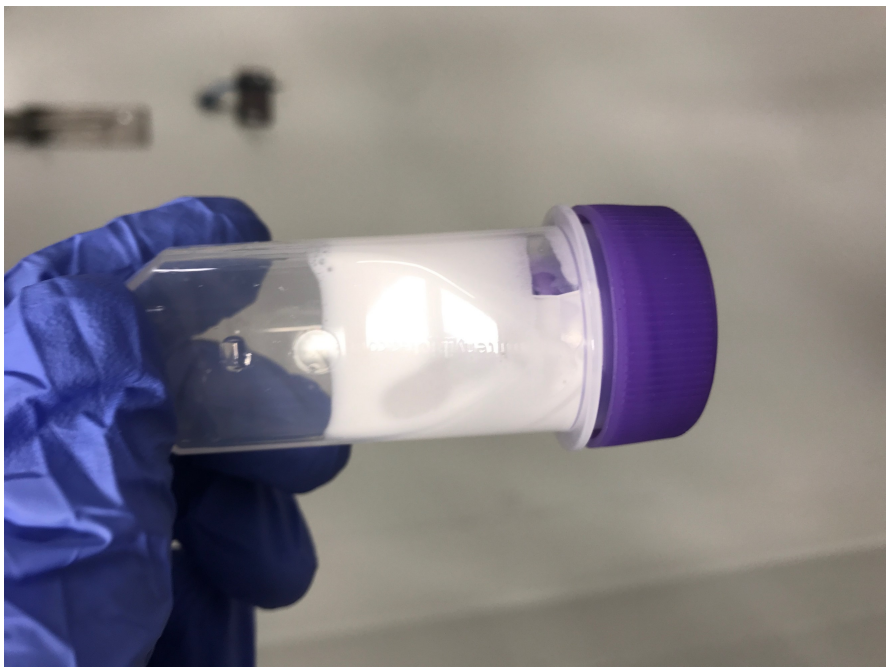
6 Run *m_lung_01 program* and *m_lung_02 program* in sequence, stopping the latter after **00:00:20** .



Foam after GentleMacs

7 Place tube **On ice** .

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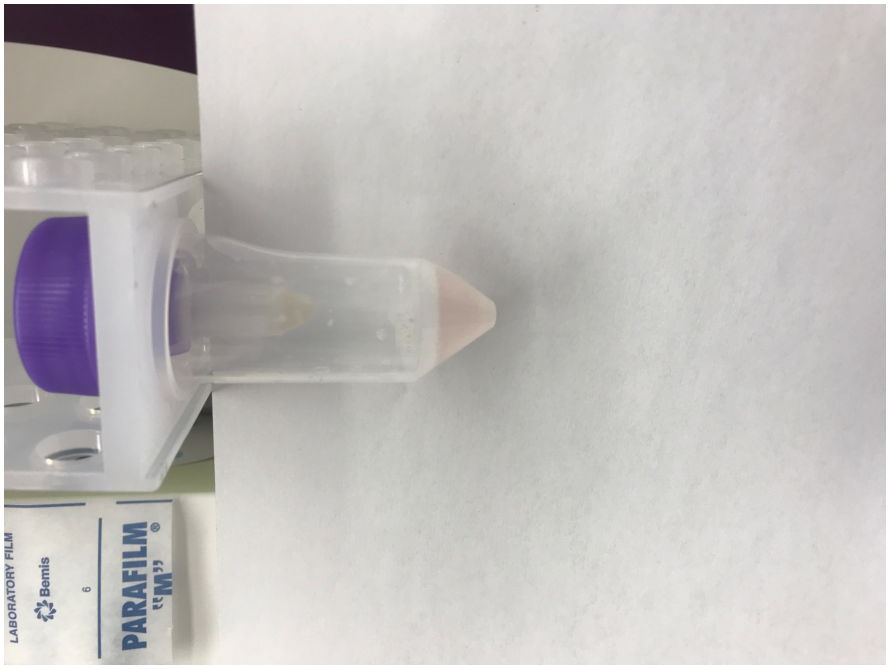
8

Reduce foam by centrifugation (**750 x g 00:01:00**).



Foam after centrifugation

9

Using a wide bore tip, pipette up and down to recover any pelleted material and pass lysate through 40 µm filter to 50 ml conical tube.

10

Wash strainer with **4 ml Wash Buffer** .

11 Pass suspension through a 5 µm strainer into 50 ml conical tube.

12

Centrifuge at **600 x g, 4°C 00:05:00** .

13 Resuspend in **500 µl Resuspension Buffer** .

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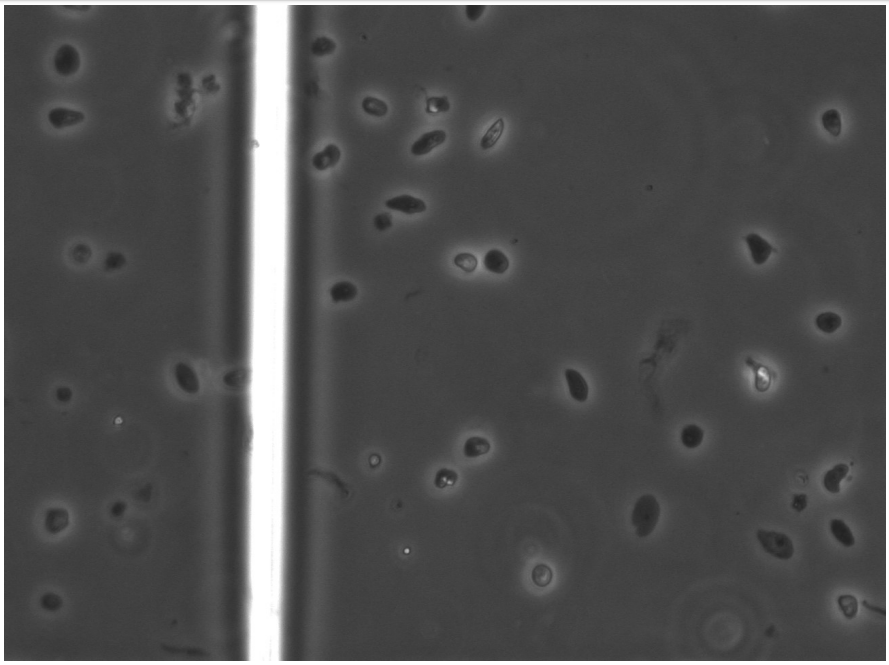
14

Count nuclei by hemocytometer and dilute to desired concentration (e.g. 10,000 per µl).

Note: it is easier to resuspend in lower volumes and dilute than to concentrate nuclei via further centrifugation.



40x Nuclear suspension with scant debris

15 Proceed to 10x Chromium.

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