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Nuclei isolation from mouse lung for single nucleus RNASeq

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Nuclei isolation from mouse lung for single nucleus RNASeq

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Human Cell Atlas Metho...

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OPEN ACCESS

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

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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.

CITATION

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

Attachments

PDF Protocol for nuclei ... 19.2MB

PDF step-by-step_Lung nu... 190KB

Materials

MATERIALS

- RNaseZap® **Thermo Scientific** **Catalog #**AM9780
- cOmplete ULTRA Tablets, Mini, EDTA-free, EASYpack **Roche** **Catalog #**05 892 791 001
- RNasin Plus Ribonuclease Inhibitors **Promega** **Catalog #**N2615
- SUPERaseIN RNase Inhibitor **Thermo Fisher Scientific** **Catalog #**AM2696
- RNase free H2O **Thermo Scientific** **Catalog #**AM9938
- Albumin, Bovine Serum, 10% Aqueous Solution, Nuclease-Free **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**126615-25ML
- Gibco™ DPBS no calcium no magnesium **Thermo Fisher Scientific** **Catalog #**14190144
- Nuclei Isolation Kit: Nuclei EZ Prep **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**NUC-101

Note

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

Equipment

gentleMACS™ C Tubes	NAME
Tissue dissociators and tubes	TYPE
gentleMACS™	BRAND
130-093-237	SKU
https://www.miltenyibiotec.com/AD-en/products/macs-sample-preparation/tissue-dissociators-and-tubes/c-tubes/gentlemacs-c-tubes.html	LINK

Equipment

gentleMACS™ Dissociator	NAME
Tissue Dissociator	TYPE
MACS	BRAND
130-093-235	SKU
https://www.miltenyibiotec.com/US-en/products/macs-sample-preparation/tissue-dissociators-and-tubes/gentlemacs-dissociator/gentlemacstm-dissociator.html	LINK

Equipment

pluriStrainer® 40 µm	NAME
Cell Strainer	TYPE
pluriSelect	BRAND
43-50040	SKU
https://www.pluriselect.com/pluristrainer-40-um-cell-strainer-51.html	LINK

Equipment

pluriStrainer® 5 µm	NAME
Cell Strainer	TYPE
pluriSelect	BRAND
43-50005	SKU
https://www.pluriselect.com/eu/pluristrainerr-5-m-cell-strainer.html#variation=150	LINK

Equipment

C-Chip™ Disposable Hemacytometers (Fuchs Rosenthal)	NAME
Counting Chamber	TYPE
INCYTO	BRAND
DHCF015	SKU
https://us.vwr.com/store/product/4618278/incyto-c-chiptm-disposable-hemacytometers-skc	LINK

Equipment

Falcon™ 15mL Polystyrene Conical Centrifuge Tubes	NAME
Centrifuge Tubes	TYPE
Falcon™	BRAND
352095	SKU
https://www.fishersci.com/shop/products/falcon-15ml-conical-centrifuge-tubes-5/p-193301	LINK

Equipment

Ambion® RNase-free 50 ml Conical Tubes	NAME
Centrifuge Tubes	TYPE
Ambion®	BRAND
AM12502	SKU
https://www.thermofisher.com/order/catalog/product/AM12502#/AM12502	LINK

Equipment

TPP 60 mm Tissue Culture Dishes	NAME
Tissue Culture Dish	TYPE
TPP	BRAND
TP93060	SKU
https://midsci.com/item/ASTPPDISH/TPP-Dishes/	LINK

Safety warnings

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

Before start

Prepare Working Solutions

Complete stock (10x)

- 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

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

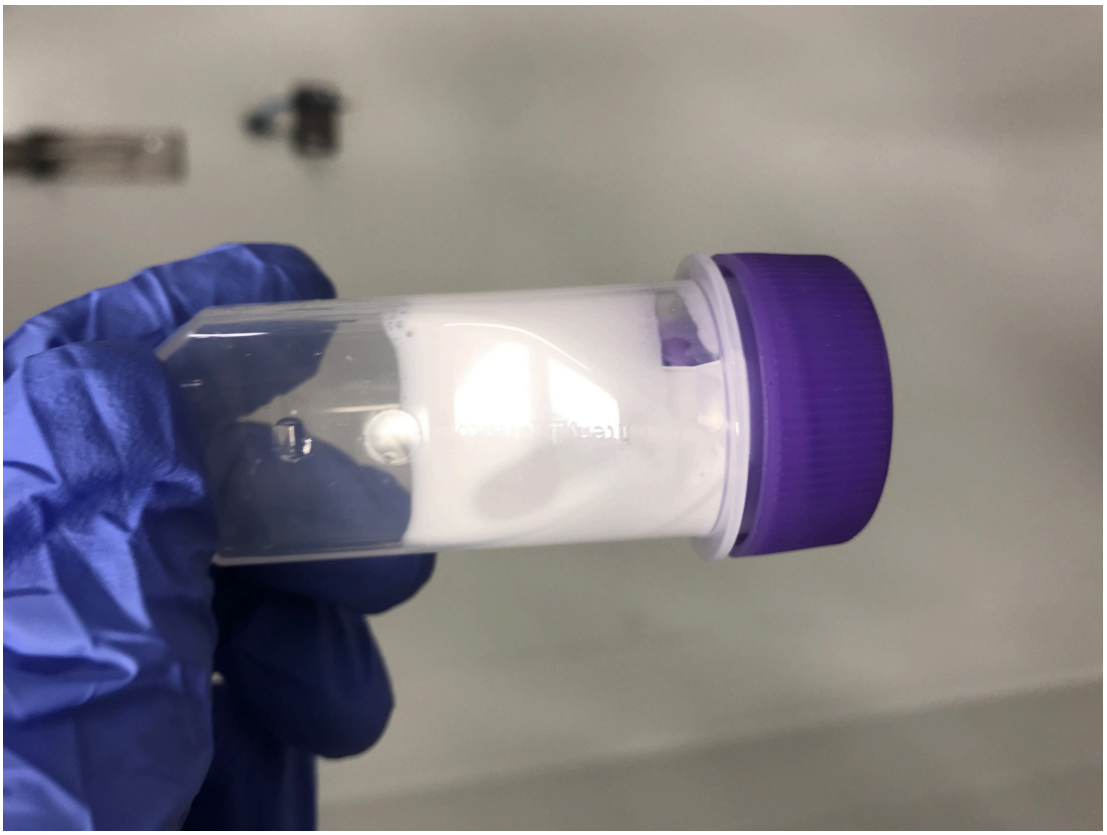
2 Remove mouse lung sample from -80 ºC freezer and trim a **~6 mm^3 piece**.

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.

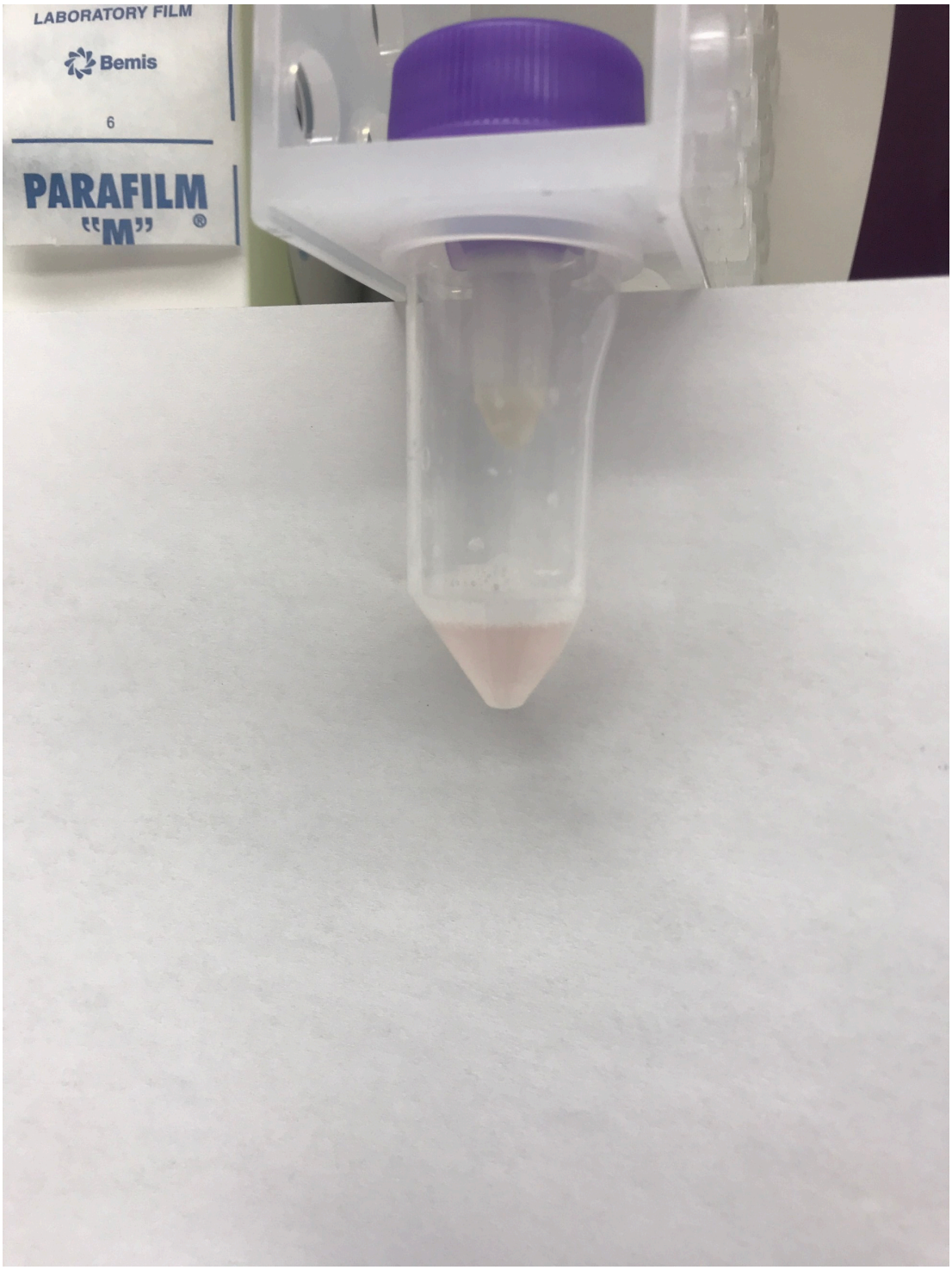
6 Run *m_lung_01 program* and *m_lung_02 program* in sequence, stopping the latter after 00:00:20 .



Foam after GentleMacs

7 Place tube On ice .

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.

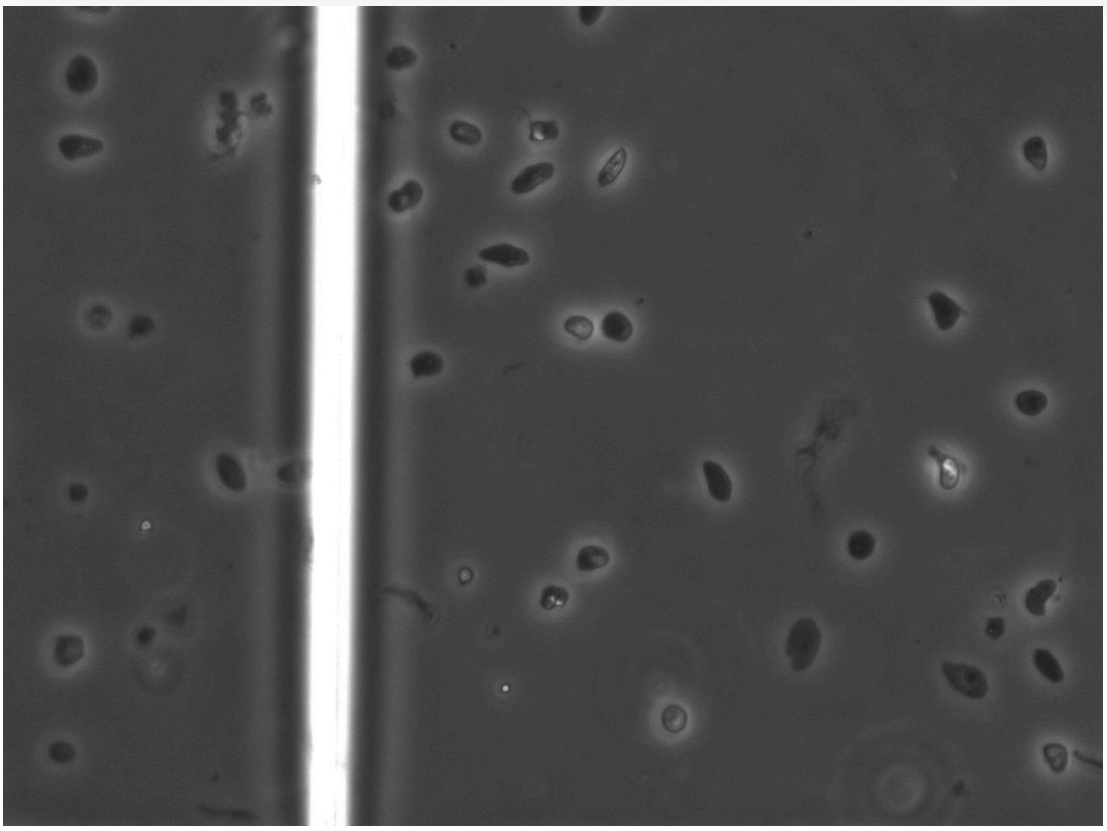
12 Centrifuge at 600 x g, 4°C, 00:05:00 .

13 Resuspend in 500 µL Resuspension Buffer .

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

Note

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.

Citations

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