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🌐 Single Cell Dissociation of Fresh Lung Tissue

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Lance Peter¹, Mei-I Chung¹, Nicholas E. Banovich¹

¹Translational Genomics Research Institute, Phoenix, AZ

Human Cell Atlas Metho...



Mei-I Chung

TGen

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Protocol status: Working

We use this protocol and it's working

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Keywords: Single Cell, Lung tissue, scRNAseq

Guidelines

All practices follow all safety guidelines regarding human tissue handling.



Materials

MATERIALS

- ✕ Collagenase Type 1 **Worthington Biochemical Corporation Catalog #LS004197**
- ✕ Collagenase Type 4 **Worthington Biochemical Corporation Catalog #LS004188**
- ✕ Neutral Protease (Dispase) **Worthington Biochemical Corporation Catalog #LS02106**
- ✕ DNase I RNase & Protease-free **Worthington Biochemical Corporation Catalog #LS006331**
- ✕ HBSS **Gibco - Thermo Fisher Scientific Catalog #14025-092**
- ✕ PBS without Ca² and Mg² **Corning Catalog #21-040-CV**
- ✕ 100um cell strainer **VWR International (Avantor) Catalog #10199-658**
- ✕ MACS SmartStrainers 30um **Miltenyi Biotec Catalog #130-098-458**
- ✕ GentleMACS C tube **Miltenyi Biotec Catalog #130-093-237**
- ✕ Bovine Albumin Fraction V (7.5% solution) **Gibco - Thermo Fisher Scientific Catalog #15260037**






Lung Dissociation

- 1 Transfer distal lung tissue to a cold petri dish and dissect into required pieces for the experiment
- 2 One piece tissue (2cm³) blot dry on disposable underpad and place to another cold petri dish. Mince the tissue into smaller pieces using scalpel. Transfer minced tissue to a GentleMACS C tube containing  7 mL of  4 °C Enzyme Mix.

Reagent	Catalog Number	Final Concentration
Collagenase Type I	LS004197	1 mg/ml
Collagenase Type 4	LS004188	1 mg/ml
Neutral Protease (Dispase)	LS02106	1 mg/ml
DNase I (RNAse & Protease-free)	LS006331	10 ug/ml
HBSS	14025-092	to 7 ml



Enzyme Mix

- 3 Place on GentleMACS Dissociator using the program  GM8.zip for  00:18:00 at  37 °C .

Note

Program installation on GentleMACS:



1. Unzip GM8.zip and place GM8 folder in a **blank** USB.
2. With machine on, insert USB
3. Select the Editor menu -> USB -> Select the program in GM8 folder -> "Save" and select desired destination folder

- 4 Add  7 mL  4 °C Inactivation Media and use a 10 ml serological pipette to titrate 5-10 times 2 ml/s. Using same pipette, pass the cell suspension through series 100 µm, 30 µm strainer into a 15 ml falcon tube.

Reagent	Catalog Number	Final Concentration
FBS	16000044	10%
PBS	21-040-CV	1X

Inactivation Media

- 5 Centrifuge 300g for  00:05:00 at  4 °C .

- 6 Remove the supernatant. Resuspend the cell pellet in  5 mL  4 °C 1X PBS 3% BSA.

Reagent	Catalog Number	Final Concentration
BSA (7.5%)	15260037	3%
PBS	21-040-CV	1X

1X PBS 3% BSA



- 7 Count cell numbers. Transfer $1 - 2 \times 10^6$ cells to a new 15 ml falcon tube.
- 8 Centrifuge 300g for 00:05:00 at 4 °C . Resuspend the cell pellet in 1 mL 4 °C 1X PBS 3% BSA.
- 9 Add 2 µL of 50 µM calcein AM to the cell suspension.
- 10 Incubate for 00:15:00 to 00:20:00 at Room temperature , protected from light.

Single Cell 5' Library

- 11 Follow 10X Genomics Chromium Single Cell 5' Library Kit. Prepare cell collection tubes containing RT Reagent Mix, Poly-dT RT Primer, Additive A, and nuclease-free water.




CG000086_ChromiumSingleCellIV_...

Reagent	Product Number	1X (µl)
RT Reagent Mix	220089	50
Poly-dT RT Primer	2000007	5.9
Additive A	220074	2.4
Nuclease-free Water	NA	16.7
Single cell suspension	NA	~15 - 16 (see next step)



RT Enzyme Mix B	2000 010 or 2000 021	10 (added last)
Total		100

- 12 Immediately after incubation, FACS (Sony SH800, 70 μm sorting chip) sorting 15,000 calcein AM positive cells into collection tubes at  4 °C .

Note

15,000 cells is about 15 - 16 μl using Sony SH800 with a 70 μm sorting chip.

We recovered 3,000 - 4,000 cells post sequencing from loading 15,000 cells. Number of cells for loading depends on targeted cell recovery and your sample recovery efficiency. Variable factors include: sorting method, time, and nozzle diameter, 10x Chromium loading efficiency, and individual sample quality. ie. Sorting 15,000 cells (70 μm nozzle) directly to the RT buffer (minus RT Enzyme Mix B) required an additional 16.7 μl nuclease-free water to reach 100 μl total reaction volume, where using a 100 μm nozzle would require less nuclease-free water.

- 13 Add 10 μl of RT Enzyme Mix B to the reaction mix + cell suspension for a total of 100 μl .
- 14 Load Chromium chip A and run Chromium controller for GEM generation.
- 15 Generate cDNA and library following the kit protocol.