Adult human lung cell dissociation (on ice) V.2

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[1]CCHMC

Version 2

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1 Works for me

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Human Cell Atlas Method Development Community

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ABSTRACT

This protocol was used to generate a single cell suspension from transbronchial biopsies from human lung. The procedure is carried out on ice, reducing artifact gene expression changes. Cell yield / mg is >5000 cells/mg with 80-90% viability (trypan blue dye exclusion). 10X scRNA-seq data is high quality (3'v3.1 and 5' v1.1) with >74% of cells retained after initial QC filtering (<20% mitochondrial gene reads, >500 genes/cell and >800 RNA molecules/cell). Expression of IER genes FOS and JUN is low in integrated, QC filtered dataset (normalized avg gene expression <3). Cell populations identified in single cell data include:

-CD8+ T
-CD4+ T
-γδ+ T
-NK
-Mast
-mDC/pDC
-B / plasma
-Monocyte
-Macrophage
-AT1 / AT2
-Ciliated
-Goblet
-Pericyte
-SMC
-Endothelial (several populations)
-Fibroblast
-Basal

If you have any questions regarding the single cell analysis thus far, please contact Andrew Potter.

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GUIDELINES

Enzyme Mix (2 x 1 mL for 13 mg tissue made up in 1.5 mL tubes)
(If digesting more than 13 mg tissue, prepare more enzyme mix as necessary)

Coll. A/Elastase/Dispase Enzyme Mix (make two tubes, each 1 mL for 13 mg starting tissue)
60 µL Collagenase A 100 mg/mL – 6 mg/mL final (Sigma, 10103578001)
100 µL Elastase 43 u/mL - 4.3 U/mL final (Worthington, LS002292)
100 µL Dispase 90 u/mL – 9 U/mL final (Worthington, LS02100)
100 µL soybean trypsin inhibitor 1 mg/mL - 100 µg/mL final (Roche, 10109886001)
5 µL 1 M CaCl2 – 5 mM final (MLS)
5 µL DNAse - 125 U/mL final (StemCell, 07469)
630 µL DPBS (no Ca, no Mg - MLS)
+13 mg tissue per 1 mL enzyme mix

SAFETY WARNINGS

Dissociation of human tissue must be performed in biosafety cabinet. Use

caution when handling tissue, and pipetting as aerosols may be generated.

Please review health safety warnings for enzymes and trypan blue, handle with caution.

BEFORE STARTING

Just prior to starting

- Prepare enzyme mixes and leave on ice.
- Cool centrifuges to 4 °C.

Enzyme Mixtures

- Collagenase, elastase and dispase enzyme stock mixes are prepared ahead of time by diluting in PBS, and frozen in 500 µL aliquots at -80 C.

- StemCell DNAse (#07469) has an activity of 2,000 U / mg. We weigh out the powder, dilute in PBS to 125 U per 5 µL, make single-use aliquots to 20-40 µL (depending on samples run at a time), and freeze at -80 C. For 10 mg of weighed powder (20,000 U of activity total), we add 800 µL PBS, mix gently, make aliquots and freeze.

10% FBS/PBS is made up with heat-inactivated, sterile filtered FBS (MLS) with Ca/Mg-free PBS.

Equipment

- Centrifuge for 1.5 mL, 15 mL conicals (preferably swinging bucket)
- Pipettes and pipet tips.
- 15 mL conicals (MLS)
- 1.5 mL tubes (MLS)
- 30 µM filters (Miltenyi, 130-098-458)
- Petri dish (MLS)
- Razor blades (MLS)
- Ice bucket w/ice
- Hemocytometer - InCyto Neubauer Improved (DHC-N01-5)

Isolate and mince tissue

1 Transport tissue in ice-cold PBS.

2 Transfer 13 mg tissue to petri dish on ice with 100 µL enzyme mix; using razor blade mince thoroughly for 2 min until large chunks are broken up finely.

00:02:00 mince on ice

Layer 1

3 Add 13 mg minced tissue to 900 µL enzyme mix (1 mL total) in 1.5 mL tube using cut p200 pipet to transfer.

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13 mg minced human lung tissue

4 Incubate on ice. Triturate 10x using 1 mL pipet set to 700 µL every 3 min (w/tip cut). Shake 3-5X to re-suspend every 2 min. 2d 2h 2m

00:03:00 triturate 10X

00:02:00 shake 3-5X

5 After 30 minutes of incubation let settle on ice 1 min. 2d 2h 1m

00:30:00 incubate on ice

00:01:00 settle one min

6 Pipet and save 80% of the supernatant (consisting of released cells), leaving undigested tissue chunks on the bottom of the tube. Add released cells (supernatant) to sterile 30 µM filter on 15 mL conical. Rinse filter w/12 mL ice-cold 10% FBS/PBS. Tap filter to ensure passage of cells.

12 mL ice-cold 10% FBS/PBS

7 Remove filter from 15 mL conical. Spin the 15 mL conical with released cells 300 g for 5 min at 4 °C. 5m

00:05:00 spin at 300 g

4 °C

8 Remove supernatant for the 15 mL conical with pelleted supernatant. Re-suspend the pellet in 14 mL ice-cold 10% FBS/PBS and leave on ice while performing next steps.

14 mL ice-cold 10% FBS / PBS

Layer 2

9 Add additional 1 mL enzyme mix to residual tissue chunks from Layer 1.

1 mL enzyme mix

10 Continue incubating on ice for 40 additional minutes (1 hr. 10 min. total). Triturate 10x using 1 mL pipet set to 700 µL every 3 min (w/tip cut if necessary). Shake 3-5X to re-suspend every 2 min. 45m

00:40:00 incubate on ice

00:03:00 triturate 10x

00:02:00 shake 3-5X

11 After 1 hr. 10 min total incubation time triturate digest mix 10X and add total digest mix (including any chunks) to a new sterile 30 µM filter on 15 mL conical.

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12 Rinse filter w/12 mL ice-cold 10% FBS/PBS.

12 mL ice-cold 10% FBS/PBS

13 Remove filter. Spin the 15 mL conicals from the first and second layer, 300 g for 5 min at 4 °C. 5m

00:05:00 spin 300 g

4 °C

RBC Lysis

14 Remove supernatant for all tubes. Re-suspend combined volume in 5 mL RBC lysis buffer. Pipet 20x to mix. Incubate on ice 5 min.

5 mL RBC lysis buffer

00:05:00 incubate on ice

15 Add 9 mL ice-cold 10% FBS/PBS to 5 mL RBC lysis buffer. Triturate and apply to sterile 30 µM filter on 15 mL conical.

9 mL ice-cold 10% FBS/PBS

16 Separate flow-through to two 15 mL conicals. Bring volume for each to 14 mL with ice-cold 10% FBS/PBS.

14 mL 10% FBS/PBS

Re-suspend and prepare for scSeq 5m

17 Spin 300 g for 5 min at 4 °C. Remove supernatant. 5m

00:05:00 spin 300 g

4 °C

18 Re-suspend cells in 100 µL ice-cold 10% FBS/PBS. Analyze viability and cell yield using a hemocytometer with trypan blue.

100 µL 10% FBS/PBS

19 Adjust cell concentration to 700-1200 cells / µL for 10x chromium.

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