MGH Harvard SenNet Processing murine trachea for paired single cell RNA-seq and mass spec

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

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ABSTRACT

Protocol for obtaining single cell suspension of murine trachea.

MATERIALS

PBS - Phosphate-Buffered Saline (10X) pH 7.4, RNase-free (Thermo Fisher Scientific; cat. no: AM9625)

1x PBS Buffer for washes and cell suspension
5ml Phosphate-Buffered
Saline (10X) pH 7.4, RNase-free (Thermo Fisher Scientific; cat. no: AM9625).
45ml Invitrogen water

Important: Do not use DPBS or other PBS buffers.

0.5% BSA in 1x PBS solution

Dissolve 0.250mg BSA in 1xPBS solution above.

DNase I (50,000x) 50,000 U/ml

Papain Stock Solution (2X)
40u/ml Papain (lyophilized, Worthington-Biochem, cat# LS001220) in EBSS (has Mg and Ca).
Dissolve 120 mg papain in 50 ml EBSS by rocking at room temp for 20min. Filter using syringe filters in the hood and aliquot in the hood in 1 ml tubes. Freeze at -20*C.

Activation Stock Solution (2X)
0.067 mM beta-mercaptoethanol, 1.1 mM EDTA, 5.5 mM Cysteine-HCl, in EBSS.

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Combine:
110ul of 500 mM EDTA
66ul 50 mM beta-mercaptoethanol (deli fridge in tissue culture room)
0.048g ysteine-HCl.H2O (powder above Brian's bench).
50ml EBSS

(Changes color to orange)
Freeze at -20*C.

RBC Lysis buffer (commercial, ThermoFisher Scientific, 00-4333-57)

Enzyme dissociation mix #2

25ul of 70kU/ml Collagenase I (in DMEM/ringers)
25ul of 50kU/ml hyluronidase (in DMEM/ringers)
50ul of 7.5kU/ml DNAse (in DMEM/ringers)
120ul of 2.5U/ml dispase (in DMEM/ringers)
400ul of 2X papain (40 u/ml) (in EBSS)
QC to 5 ml of DMEM (has Ca and Mg, so good to use).

Note:
Can scale down from 5ml for the number of tracheas processed.

100% FBS
Thaw in advance in water bath.

DMSO

Dissection

1 Euthanize mice by CO2 method.

2 Perfuse lungs via right ventricle.

3 Dissect out trachea and lungs and place in HBSS on ice.

4 Clean up the tracheas under the dissection microscope.

5 Bi-sect the tracheas along the ventral side.

Single cell suspension

6 Incubate each trachea in 500ul enzyme dissociation mix #1 for 30min with rotation at 37*C.

Enzyme dissociation mix #1
1ml of papain
1ml of activation solution
20ul Dnase I

7 Cut off the tip of p1000 tip and pipet trachea up and down 10 times. Should get stringy.

8 Spin down for 5 min at 2000g. This will pellet both single cells and the husk.

9 Using a pipet (not the vacuum), remove as much supernatant as possible without being a hero.

10 Resuspend cells and husk in 500ul enzyme mix #2 per trachea for 20 minutes at 37C with rocking.

Enzyme dissociation mix #2
25ul of 70kU/ml Collagenase I (in DMEM/ringers)
25ul of 50kU/ml hyluronidase (in DMEM/ringers)
50ul of 7.5kU/ml DNAse (in DMEM/ringers)
120ul of 2.5U/ml dispase (in DMEM/ringers)
400ul of 2X papain (40 u/ml) (in EBSS)
QC to 5 ml of DMEM (has Ca and Mg, so good to use).

11 Cut off the tip of p1000 tip and pipet trachea up and down 10 times. Husk will be mostly gone.

12 Spike in 55ul FBS for 10% FBS final concentration to inactivate papain and mix well.

13 Strain cells through 100um strainer.

14 Centrifuge at 8000 × g for 2.5 min, discard the supernatant.

15 Add 300 uL of RBC lysis buffer per trachea and incubate for 60s on the bench.

16 Add 1 mL of EBSS to stop the lysing, centrifuge at 8000 × g for 2.5 min, discard the supernatant.

17 Resuspend with 200 uL of 0.5% BSA in PBS. Keep on ice.

Count cells with heamocytometer

18 Count two samples each per trachea. We routinely recover 250,000-350,000 cells with this protocol.

Prepare samples for scRNA-seq and scMS

19 Split each tracheal sample into 50,000 cell aliquots, according to the cell counts determined above.

20 Keep one aliquot on ice for Andrew to pick up. This is the scRNA-seq sample.

21 For the remaining 3-4 aliquots, spin down at 8000 × g for 5 min, discard the supernatant and resuspend in 90% FBS, 10% DMSO and freeze at -80*C. These are the scMS samples.

scRNA library preparation

22 Prepare according to manufacturer's protocol:

CG000204_ChromiumNextGEMSingleCell3_v3.1_Rev_D.pdf