Adult mouse liver dissociation (on ice)

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Abstract

This protocol is used to dissociate adult (10 week) CD-1 mouse liver "on ice", preserving authentic gene expression profiles. The first layer consists of 3.5 hours rotation at 4 °C in 0.25% trypsin, and the second layer consists of 15 min. in *bacillus licheniformis*, with trituration on ice. The yield is 5000 cells/mg with 97% viability (as measured by trypan blue).

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Guidelines

Trypsin Enzyme Mix

500 µL DPBS (ThermoFisher cat. # 14190) / BSA 0.1% (0.05% BSA/DPBS final conc.)

500 µL Trypsin 0.5%/EDTA – 0.25% final conc. (ThermoFisher, cat. # 15400054)

Bacillus Licheniformis Enzyme Mix

899 µL DPBS (ThermoFisher, cat. # 14190)

1 µL 0.5 M EDTA (0.5 mM final) (Sigma, A8806)

100 µL *Bacillus Licheniformis* 100 mg/mL - 10 mg/mL final conc. (Sigma, P5380)

Preparing enzymes:

The *bacillus licheniformis* is made up in DPBS (#14190). It is aliquoted and stored at -80 ºC at 100 mg/mL in 100 µL aliquots.

The trypsin is aliquoted and stored at -20 ºC.

Required Equipment & Consumables:

Refrigerated centrifuge

Fisher tube rotator, cat. # 88-861-051 (or similar rotation device)

Pipettes and pipet tips (MLS)
15, 50 ml Conicals (MLS)
1.5 mL tubes (MLS)
30 µM filters (MACS SmartStrainers, 130-098-458)

Petri dishes (MLS)
Razor blades (MLS)
Ice bucket w/ice (MLS)
Hemocytometers - InCyto Neubauer Improved (DHC-NO1-5)

Required reagents:

Red Blood Cell Lysis Buffer - Sigma (R7757)

The protocol workflow is as follows:

1. Isolate liver
2. First layer
3. Second layer
4. Preparing cells for Chromium/DropSeq

Before start

-Prepare enzyme mixes and leave on ice.

-Cool centrifuges to 4 °C.

-Isolate and transport tissue in ice-cold DPBS.

Protocol

Step 1.
Dissect out liver tissue and place in ice-cold PBS.

Step 2.
Mince tissue thoroughly on petri dish on ice (2 min) until fine paste.

DURATION
00:02:00 : mince on ice

Step 3.
Add 18 mg tissue to 1 mL trypsin enzyme mix. Shake tube vigorously to re-suspend tissue.

AMOUNT
18 mg : minced tissue

Step 4.

Leave digest mix rotating at 4 °C for 3.5 hrs on Fisher tube rotator. Every 45 min (4X total), stop rotation briefly and shake tube vigorously to re-suspend tissue.

DURATION
03:30:00 : rotate at 4 °C
DURATION
00:45:00 : shake vigorously

Step 5.

After 3.5 hours, transfer digest mix to 15 mL conical & add 10 mL ice-cold DPBS to dilute trypsin mix. Re-suspend digest mix.

AMOUNT
10 ml : ice-cold DPBS

Step 6.

Spin 200 g for 5 min at 4 °C. Remove supernatant.

DURATION
00:05:00 : spin 200 g

Step 7.

Re-suspend cells and tissue in 1 mL ice-cold DPBS and transfer to 1.5 mL tube. Let chunks settle one minute on ice (released cells should remain in supernatant).

DURATION
00:01:00 : let chunks settle on ice

Step 8.

After tissue has settled to bottom of tube, transfer 75% of supernatant, containing released cells onto 30 µM filter on 50 mL conical. Rinse filter with 6 mL ice-cold PBS/BSA 0.04%. Save filter and flow-through for next steps.

AMOUNT
750 µl : pipet off supernatant
AMOUNT
6 ml : ice-cold PBS/BSA 0.04%

Step 9.

To tissue clumps at bottom of tube, add 1 mL bacillus licheniformis enzyme mix. Triturate 10X.

AMOUNT
1 ml : bacillus licheniformis enzyme mix

Step 10.

Continue incubating on ice in bacillus licheniformis enzyme mix. Shake every minute and triturate 10X every 2 min for 15 additional minutes (3 hr. 45 min total time) until the majority of clumps are broken up.

DURATION
00:15:00 : triturate on ice to break up remaining chunks

Step 11.

Transfer entire volume of digest mix to the same 30 µM filter. Rinse filter with 5 mL ice-cold PBS/BSA 0.04%.

AMOUNT
5 ml : ice-cold PBS/BSA 0.04%

Step 12.

Transfer flow-through to 15 mL conical.

Step 13.

Spin 200 g for 5 min at 4 °C. Remove supernatant (down to 100 µL) and leave in 15 mL conical.

Step 14.

Add 1 mL RBC lysis buffer; triturate 20X; let sit 2 min. on ice.

AMOUNT
1 ml : RBC lysis buffer

DURATION
00:02:00 : incubate on ice

Step 15.

After 2 min., add 5 mL ice-cold PBS/BSA 0.04% and re-suspend cells.

AMOUNT
5 ml : ice-cold PBS/BSA 0.04%

Step 16.

Add re-suspended cells to new 30 µM filter on 50 mL conical; rinse filter with 7 mL ice-cold PBS/BSA 0.04%. Transfer flow-through to 15 mL conical.

AMOUNT
7 ml : ice-cold PBS/BSA 0.04%

Step 17.

Spin 1800 RPM for 3 minutes at 4 °C to pellet cells and leave the majority of debris in supernatant.

DURATION
00:03:00 : spin 1800 RPM

Step 18.

Remove supernatant; re-suspend cells in 100 µL ice-cold PBS/BSA 0.04% and analyze using hemocytometer with trypan blue. Adjust cell concentration to 1,000 cells/µL for Chromium or 100 cells/µL for DropSeq.

AMOUNT
100 µl : ice-cold PBS/BSA 0.04%