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Working

Adult mouse liver dissociation (on ice)

Version 2

Andrew Potter[1]

[1]CCHMC

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Human Cell Atlas Method Development Community

Andrew Potter

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).

DOCX

Liver cell dissociation 7.31.18.docx

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

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

MATERIALS

NAME	CATALOG #	VENDOR
RBC Lysis Buffer	R7757	Sigma

BEFORE STARTING

-Prepare enzyme mixes and leave on ice.
-Cool centrifuges to 4 °C.
-Isolate and transport tissue in ice-cold DPBS.

Isolate tissue

1 Dissect out liver tissue and immerse/transport in ice-cold PBS.

2 Using sterile forceps, place tissue on petri dish on ice. Remove excess DPBS using pipet. Mince tissue thoroughly on petri dish on ice (~2 min) until fine paste. Manipulate tissue with forceps while mincing with razor blade.

00:02:00 mince on ice

First layer of dissociation

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

18 mg minced tissue

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.

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

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.

10 ml ice-cold DPBS

6 Spin 300 g for 5 min at 4 °C to pellet cells and tissue chunks. Remove supernatant.

00:05:00 spin 300 g **4 °C**

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).

00:01:00 let chunks settle on ice

8 After tissue chunks have 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.

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

Second layer of dissociation

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

1 ml bacillus licheniformis enzyme mix

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.

00:15:00 incubate on ice **00:02:00 triturate 10x every 2 min**

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

5 ml ice-cold PBS/BSA 0.04%

12 Re-suspend and transfer flow-through containing cells to 15 mL conical.

13 Spin 300 g for 5 min at 4 °C. Remove supernatant (down to ~100 µL) and leave in 15 mL conical.

00:05:00 300 g for 5 min **4 °C**

RBC Lysis

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

1 ml RBC lysis buffer

00:02:00 incubate on ice

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

5 ml ice-cold PBS/BSA 0.04%

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.

7 ml ice-cold PBS/BSA 0.04%

Preparing for single cell analysis

17 Spin 1800 RPM (low-speed spin) for 3 minutes at 4 °C to pellet cells and leave the majority of debris in supernatant.

00:03:00 spin 1800 RPM **4 °C**

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.

100 µl ice-cold PBS/BSA 0.04%