VERSION 2

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 We use this protocol and it's working

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Adult mouse liver dissociation (on ice) V.2

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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).

ATTACHMENTS

[Liver cell dissociation 7.31.18.docx](#)

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 °C at 100 mg/mL in 100 µL aliquots.

The trypsin is aliquoted and stored at -20 °C.

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

MATERIALS

RBC Lysis Buffer **Sigma Catalog #R7757**

BEFORE START INSTRUCTIONS

- 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 μ M 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 μ L 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 μ M 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 μ L) 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 μ M 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 μ L ice-cold PBS/BSA 0.04% and analyze using hemocytometer with trypan blue. Adjust cell concentration to 1,000 cells/ μ L for Chromium or 100 cells/ μ L for DropSeq.

100 μ L ice-cold PBS/BSA 0.04%