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Mouse Liver Single-Cell Dissociation with Cold Protease

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ABSTRACT

This protocol is used to dissociate murine liver cells at 4 °C in an effort to preserve transcriptomic expression profiles in cells to be used for single-cell RNA sequencing and single-cell ATAC sequencing. An expedited protocol length limits the time of exposure to dissociative solutions in order to retain natural cellular distribution and viability while limiting transcriptomic changes arising from injury response, especially in fragile cell populations. This protocol has been tested and works in mice aged 2-4 weeks, dissociating cells from the left lobe for single-cell RNA sequencing using the 10x Chromium system.

This project was supported in part by NIH P30 DK078392 (Gene Analysis Core) of the Digestive Diseases Research Core Center in Cincinnati, R01DK107553, and the Cincinnati Pediatric Cell Atlas Center funded by Cincinnati Children's Research Foundation to Stacey S. Huppert

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GUIDELINES

Bacillus licheniformis enzyme mix

The Bacillus licheniformis mix is made up in DPBS at a concentration of 100mg/mL and stored at -80 °C in 100µL aliquots

To make 10mL of 1mg/mL Bacillus licheniformis mix:
9900µL DPBS
100µL Bacillus licheniformis 100mg/mL mix

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10x PBS (1L)
80g NaCl
2g KCl
11.5g Na_2HPO_4 $7H_2O$
2g KH_2PO_4
1L diWater

1x PBS for Flush
Make up 5 mL of 1x PBS by diluting 500µL 10x PBS in 4.5mL MilliQ water

Required Equipment
Refrigerated centrifuge
Tube rotator (or similar device)

MATERIALS

NAME	CATALOG #	VENDOR
Protease from Bacillus Licheniformis	P5380	Sigma
3 mL syringes	BD #309657	BD Biosciences
Falcon™ Cell Strainers, Mesh size: 70um; white	087712	Thermo Fisher
William's E Medium, no glutamine	12551032	Thermo Fisher
DPBS, calcium, magnesium	14040133	Thermo Fisher
100um Cell Strainer	22363549	Fisher Scientific
EqualFetal Bovine Serum	EF-0500-A	Atlas Biologicals
PrecisionGlide Needle 23G	BD #305120	BD Biosciences
BD Intramedic™ Polyethylene Tubing PE-50	BD #427516	Fisher Scientific

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BEFORE STARTING

Prepare essential solutions and leave on ice
Cool centrifuges to 4 °C
To make cannulation apparatus:

- Cut ~12 inch length of PE-50 tubing and make an angled cut at one end with a razor blade to serve as the cannulation tip.
- Carefully insert 23-gauge needle tip into opposite end of tubing, making sure not to poke a hole in tubing
- If a smaller cannula is needed to fit into the portal vein, the PE-50 can be warmed by rolling between fingers and then stretched by pulling. Additionally, PE-10 can be used for very small vessels.

Tissue Isolation 20m

1 Anesthetize mouse

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1.1 Fill syringe with 1x PBS and twist on to needle with attached cannulation apparatus. Flick syringe and push plunger to ensure 1x PBS has filled the tubing and there are no air bubbles in the syringe or tubing

1.2 Open abdominal cavity and locate main trunk of portal vein. Make a small incision to allow for insertion of angled tip of cannulation apparatus without cutting all the way through portal vein

1.3 Make incision in inferior vena cava to allow blood and perfusing solutions to flow out

2 Cannulate the portal vein and flush 3-5mL ice cold 1x PBS through portal vein until liver tissue is sufficiently cleared of blood

5 mL ice-cold 1x PBS

3 Switch PBS syringe out for one with Bacillus licheniformis mixture while keeping the portal vein cannulated. Ensure no bubbles are in the system and then perfuse 3-5mL ice cold 1mg/mL Bacillus licheniformis mixture through the portal vein

5 mL ice-cold Bacillus licheniformis enzyme mix

4 Dissect out desired liver tissue and mince thoroughly with razor blade in petri dish with an additional 1mL Bacillus licheniformis mix; start timer for 1 hour

1 mL ice-cold Bacillus licheniformis enzyme mix

Dissociation and Filtration 1h 5m

5 Transfer tissue-enzyme slurry to 5mL capped tube and incubate, rotate, or rock at 4 °C until timer reaches 1 hour

01:00:00 at 4 °C

6 Mince slurry again with razor blade, making sure to separate or scrape larger chunks to release the maximum number of cells

7 Pass slurry through a 100µM filter into a 50mL conical tube

8 Bring volume in tube up to 10mL by slowly passing ice cold Williams' E media with 20% FBS through filter to dislodge any sticking cells and stop the enzyme activity

10 mL Williams" E 20% FBS with slurry

Centrifugation and Resuspension 15m

9 Centrifuge cells at 250g for 5min at 4 °C

00:05:00 centrifuge 250g at 4 °C

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10 Discard supernatant and resuspend pellet in 5mL Williams E with 10% FBS

5 mL resuspend Williams' E 10% FBS

11 Filter suspension through a 70µM filter into a new 50mL conical tube; rinse filter with an additional 5mL of media

5 mL Williams' E 10% FBS

12 Repeat steps 9-10

00:05:00 centrifuge 250g at 4 °C

5 mL resuspend Williams' E 10% FBS

Final resuspension volume may be greater or less than stated and should be judged accordingly based on pellet size

Quality Control 10m

13 Analyze cell morphology using hemocytometer and Trypan blue

Viable hepatocytes tend to take up trace amounts of Trypan blue

14 Adjust cell concentration as needed (700-1200 cells/µL for 10x Chromium system)

1000 cells/µL

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