ABSTRACT
Analyzing the mouse liver requires optimal cell recovery from the tissue in terms of quality (viability) and quantity. Harsh dissociation methods can damage fragile cell populations, resulting in low viability and difficulty in cell culture. We have developed a gentle surgical and enzymatic technique based on the two-step collagenase perfusion protocol for single-cell analysis and primary cell culture.

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Preparation

1. **Anesthetize** mouse with inhalational anesthesia (4-5% isofluorane) in the induction chamber.

2. **Heparinize** the animal intraperitoneally by injecting 5 units/g (body weight) of heparin:

   Preventing blood clotting improves cell recovery.
Once the animal is anesthetized, transfer it onto a homeothermic warming pad. The animal will receive 1-3% isofluorane via face-mask inhalation for the remaining steps.

Prepare the abdomen for surgery: shaved with a trimmer, prepared with iodine-based solution and alcohol-based solution, and draped in a sterile fashion.

Laparotomy

Midline incision: cut open the abdomen from the pubic symphysis to the xiphoid process. Flip over the intestines to locate the liver and its portal vein.

Please refer to Figure 14.4.1 of the following publication:

https://doi.org/10.1002/0471140856.tx1404s14

Cannulation

Cannulate the portal vein with the 22-gauge catheter. Secure it cannulation by suture or a micro serrefine.

Insite™ Autoguard™ BC
Intravenous Catheter
BD 382523
Non-winged, blue, 22 gauge, 1 inch, 0.9 × 25 mm

Schwartz Micro Serrefine
Micro Serrefine
Fine Science Tools 18052-03
Sharp Bend
Collect blood: collect blood from the inferior vena cava (IVC) with a 20-gauge needle.

Cut the IVC and proceed to the next step immediately. Cutting the IVC allows blood and perfusate to drain.

Perfusion

Perfuse liver with calcium-free buffer without collagenase: 10 ml of Hanks’ Balanced Salt Solution (HBSS) without calcium or magnesium + 0.5 Millimolar (mM) EGTA at a flow rate of 2 mL/min until fluid drained from the IVC is no longer red (containing blood). This is done with a pump with no recirculation.

This flow rate (2 mL/min) is slower than other liver perfusion protocols. We found that a slower perfusion rate results in hepatocytes with higher quality and quantity and therefore suitable for analyses such as single-cell RNA sequencing.

Model EP-1 Econo Pump
Peristaltic Pump
Bio-Rad 7318140

Perfuse liver with calcium-containing buffer with collagenase: 10 mL of Hanks’ Balanced Salt Solution (HBSS) with calcium and magnesium + 0.1 mg/mL type IV collagenase at the same flow rate (2 mL/min). This is done with the same pump with no recirculation.

Collagenase from Clostridium histolyticum Millipore
Sigma Catalog #C5138

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A well-perfused liver shows drastic discoloration due to the lack of blood. A well-dissociated liver shows white patches.

**Harvest**

11 **Excise** the dissociated liver out of the animal and place it in DMEM containing 10% FBS, which inactivates the collagenase enzyme.

- Dulbecco’s Modified Eagle’s Medium - high glucose Millipore
- Sigma Catalog #D5796

**Cell Isolation**

12 **Gently cut** the excised liver with a scalpel to release the dissociated cells.

Good dissociation can be visualized by abundance of cells released (in pink) with little cutting. Shake the tissue gently to release cells. Avoid tearing the tissue with the scalpel (horizontal movement); only cut the tissue vertically.

13 **Filter** dissociated cells with a 70 µm filter:

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Cell Enrichment

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**Enrich dissociated cells for hepatocytes** by centrifugation **50 x g**  & **Room temperature** for 5 minutes.
The supernatant can be further purified in the next step. Resuspend the pellet (hepatocyte-rich) with DMEM.

15

**Enrich supernatant for non-parenchymal cells (NPC)** by centrifugation **400 x g**  & **Room temperature** for 5 minutes. Discard the supernatant. Resuspend the pellet (NPC-rich) with DMEM.