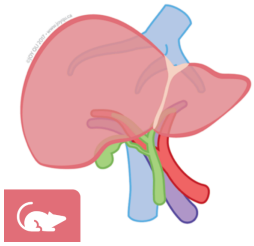
Nov 24, 2020

Mouse 2- Step Collagenase Liver Perfusion protocol



DOI
dx.doi.org/10.17504/protocols.io.53qg8mw

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Protocol Citation: Michael L Cheng, Xue-Zhong Ma, Chao Jiang, Ian McGilvray, Sonya Macparland 2020. Mouse 2- Step Collagenase Liver Perfusion protocol . **protocols.io https://dx.doi.org/10.17504/protocols.io.53qg8mw**

Manuscript citation:

Protocol status: Working
We use this protocol and it's working

Created: August 01, 2019

Last Modified: November 24, 2020

Protocol Integer ID: 26448

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.

Protocol materials

- Dulbecco's Modified Eagle's Medium - high glucose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D5796
- Heparin LEO® **LEO Pharma Catalog #**006174-09
- Collagenase from Clostridium histolyticum **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**C5138

Preparation

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

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

Note

Preventing blood clotting improves cell recovery.

Heparin LEO® **LEO Pharma Catalog** #006174-09

3 **Once the animal is anesthetized, transfer** it onto a homeothermic warming pad. The animal will receive 1-3% isofluorane via face-mask inhalation fo the remaining steps.

4 **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

5 **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:

CITATION

Froh M, Konno A, Thurman RG (2003). Isolation of liver Kupffer cells.. Current protocols in toxicology.
LINK
https://doi.org/10.1002/0471140856.tx1404s14

Cannulation

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

Equipment

Insyte™ Autoguard™ BC	NAME
Intravenous Catheter	TYPE
BD	BRAND
382523	SKU
https://www.bd.com/en-ca/offerings/capabilities/infusion-therapy/iv-catheters/bd-insyte-autoguard-bc-shielded-iv-catheter-with-blood-control-technology	LINK
Non-winged, blue, 22 gauge, 1 inch, 0.9 × 25 mm	SPECIFICATIONS

Equipment

Schwartz Micro Serrefine	NAME
Micro Serrefine	TYPE
Fine Science Tools	BRAND
18052-03	SKU
https://www.finescience.com/en-US/Products/Vascular-Instruments/Clamps-Occluders/Schwartz-Micro-Serrefines/18052-03	LINK
Sharp Bend	SPECIFICATIONS

7 **Collect blood:** collect blood from the inferior vena cava (IVC) with a 20-gauge needle.

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

Perfusion

9 **Perfuse liver with calcium-free buffer without collagenase:** 10 mL of Hanks' Balanced Salt Solution (HBSS) **without** calcium or magnesium + [M] 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. 5m

Note

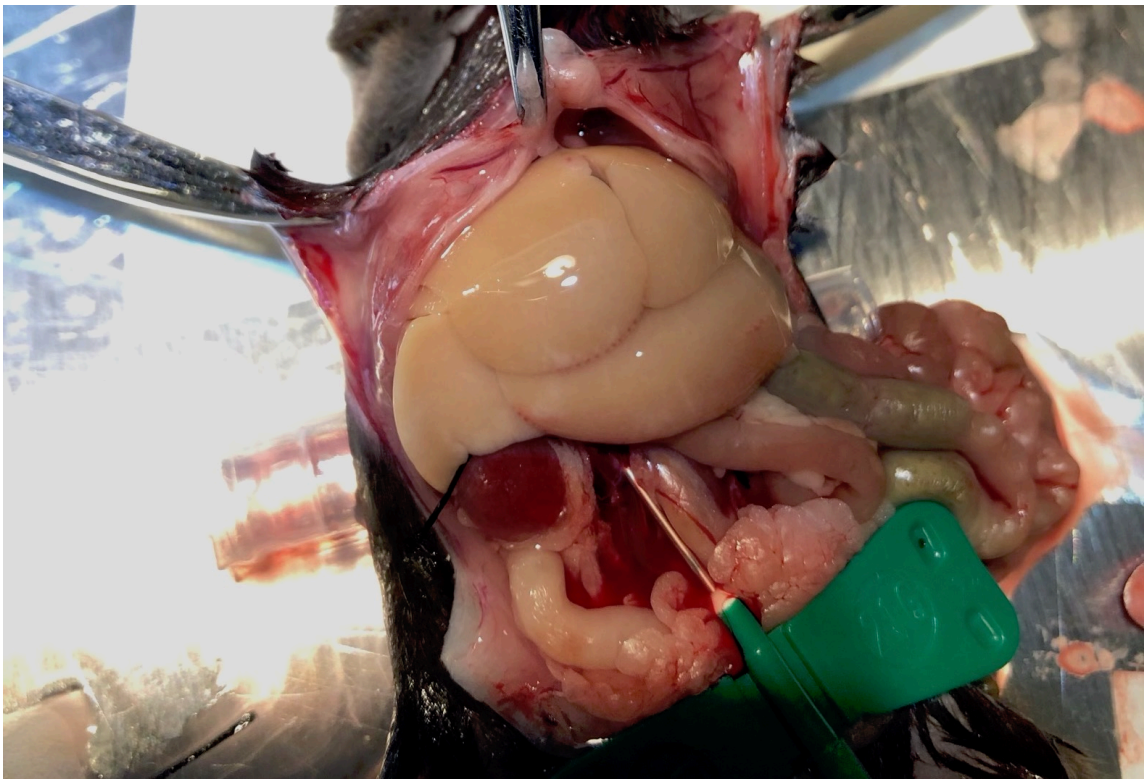
This flow rate **(2 mL/min)** is slower than other liver perfusion protocols. We found that a slower perfusion rate results in hepatocytes with higher quality and quantity and therefore suitable for analyses such as single-cell RNA sequencing.

Equipment

NAME	TYPE	BRAND	SKU	LINK
Model EP-1 Econo Pump	Peristaltic Pump	Bio-Rad	7318140	http://www.bio-rad.com/en-ca/product/model-ep-1-econo-pump?ID=6ee41e9b-5ac5-449c-ae7d-578dca8ed6cf

10 **Perfuse liver with calcium-containing buffer with collagenase:** 10 mL of Hanks' Balanced Salt Solution (HBSS) **with** calcium and magnesium + [M] 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. 5m

Collagenase from Clostridium histolyticum **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**C5138



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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D5796

Cell Isolation

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

Note

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:

Equipment

70 µm Cell Strainer	NAME
Cell Strainer	TYPE
Falcon	BRAND
352350	SKU
https://ecatalog.corning.com/life-sciences/b2c/US/en/Cell-Culture/Cell-Culture-Accessories/Cell-Strainers/Falcon®-Cell-Strainers/p/352350?clear=true	LINK
White, Sterile, Individually Packaged	SPECIFICATIONS

Cell Enrichment

14 **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. 5m

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. 5m

Citations

Step 5

Froh M, Konno A, Thurman RG. Isolation of liver Kupffer cells.
https://doi.org/10.1002/0471140856.tx1404s14