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High-quality single-cell RNA-sequencing data of liver cells, especially hepatocytes, are challenging due to cell death associated with hepatocyte isolation using fluorescence-activated cell sorting (FACS). Here, we present a protocol to obtain viable hepatocytes and nonparenchymal liver cells for scRNA-seq, using centrifugation. We detail steps for liver wash and enzyme perfusion, followed by in vitro dissociation of liver cells and gradient centrifugation. We further describe hepatocyte cells harvesting for subsequent viability check and scRNA-seq.

Publisher’s note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.
Protocol to obtain high-quality single-cell RNA-sequencing data from mouse liver cells using centrifugation

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https://doi.org/10.1016/j.xpro.2022.101824

SUMMARY

High-quality single-cell RNA-sequencing data of liver cells, especially hepatocytes, are challenging due to cell death associated with hepatocyte isolation using fluorescence-activated cell sorting (FACS). Here, we present a protocol to obtain viable hepatocytes and nonparenchymal liver cells for scRNA-seq, using centrifugation. We detail steps for liver wash and enzyme perfusion, followed by \textit{in vitro} dissociation of liver cells and gradient centrifugation. We further describe hepatocytes harvesting for subsequent viability check and scRNA-seq. For complete details on the use and execution of this protocol, please refer to Wang et al. (2021) and Mederacke et al. (2015).

BEFORE YOU BEGIN

Institutional permissions (if applicable)
All animal studies were approved and conducted under the oversight of the UT Southwestern Institutional Animal Care and Use Committee.

Prepare stock buffers

© Timing: 4 h

1. EGTA Stock Buffer.
   a. Make EGTA Stock Buffer as described in the materials and equipment in a 2-L glass beaker.
   b. Adjust pH to 7.35.
   c. Filter reagent through a 0.22 μm polyether sulfone (PES) membrane filter.
   d. Store reagent at 4°C. The maximum storage time is 3 months.

2. Enzyme Stock Buffer.
   a. Make Enzyme Stock Buffer as described in the materials and equipment in a 2-L glass beaker.
   b. Adjust pH to 7.35.
   c. Filter reagent through a 0.22 μm PES membrane filter.
   d. Store reagent at 4°C. The maximum storage time is 3 months.

3. Grey’s A Stock Buffer.
   a. Make Grey’s A Stock Buffer as described in the materials and equipment in a 2-L glass beaker.
   b. Adjust pH to 7.35.
   c. Filter reagent through a 0.22 μm PES membrane filter.
   d. Store reagent at 4°C. The maximum storage time is 3 months.

4. DNase Stock Solution.
a. Dissolve 100 mg DNase powder in 50 mL Grey’s balanced salt solution (GBSS) in a 50-mL centrifuge tube.
b. Mix well by vortexing at the maximum speed. The concentration of DNase Stock Solution is 2 mg/mL.
c. Aliquot 1.0 mL of the 50 mL DNase Stock Solution into fifty 1.5 mL Eppendorf tubes for storage.
d. Store the DNase Stock Solution in −20°C for a maximum of 6 months.

**Prepare working solutions**

© Timing: 1 h

5. EGTA Working Buffer.
   a. Aliquot 35 mL EGTA Stock Buffer into a 50-mL centrifuge tube for perfusion of 1 mouse liver.
   b. Pre-warm the 50-mL centrifuge tube containing 35 mL EGTA Stock Buffer in a 39°C water bath for 15 min before liver perfusion.
   c. Keep EGTA Working Buffer in 39°C water bath during the perfusion for a maximum of 2 h.

6. Pronase Working Buffer.
   a. Prepare 35 mL Pronase Working Buffer as described in the materials and equipment in a 50-mL centrifuge tube for perfusion of 1 mouse liver.
   b. Vortex the mixture at maximum speed to fully dissolve the enzyme.
   c. Filter the solution through a Millex-GV Syringe Filter Unit, 0.22 μm polyvinylidene fluoride (PVDF) membrane filter.
   d. Pre-warm the 35 mL Pronase Working Buffer in 39°C water bath for 15 min before perfusion.
   e. Keep Pronase Working Buffer in 39°C water bath during the perfusion for a maximum of 2 h.

7. Collagenase D Working Buffer.
   a. Prepare 40 mL Collagenase D Working Buffer as described in the materials and equipment in a 50-mL centrifuge tube for perfusion of 1 mouse liver.
   b. Vortex the mixture at maximum speed to fully dissolve the enzyme.
   c. Filter the solution through a Millex-GV Syringe Filter Unit, 0.22 μm PVDF membrane filter.
   d. Pre-warm the 40 mL Collagenase D Working Buffer in 39°C water bath for 15 min before perfusion.
   e. Keep Collagenase D Working Buffer in 39°C water bath during the perfusion for a maximum of 2 h.

8. Liver Dissociation Buffer.
   a. Prepare 50 mL Liver Dissociation Buffer in a 50 mL centrifuge tube.
   b. Vortex the mixture at maximum speed to fully dissolve the enzymes.
   c. Pre-warm the 50 mL Liver Dissociation Buffer in 39°C water bath for 15 min before perfusion.
   d. Keep Liver Dissociation Buffer in 39°C water bath during the perfusion for a maximum of 2 h.

9. Thaw 1 mL DNase Stock Solution for later use at 4°C for a maximum of 4 h.

10. Nycodenz Buffer.
    a. Measure 4.94 g Nycodenz powder and transfer to a 50 mL centrifuge tube.
    b. Prepare 18 mL Nycodenz Buffer by first adding 15 mL Grey’s A Stock Buffer to the 50 mL centrifuge tube containing Nycodenz powder.
    c. Vortex at maximum speed until most of the Nycodenz powder has been dissolved. The final working concentration of the Nycodenz buffer is 0.274 g/mL.
    d. Then, add an additional 3-mL Grey’s A Stock Buffer to the mixture.
    e. Vortex at maximum speed to fully dissolve the mixture.
    f. Store prepared Nycodenz Buffer for later use at 4°C for a maximum of 4 h.

11. Set up perfusion pump.
    a. Assemble perfusion pump, Luer Lock Connect and polyethylene tubing.
    b. Insert the polyethylene tube in EGTA Working Buffer and make a bubble trap. See Figure 1. The other end of Luer Lock Connect will connect to 18-gauge catheter later.
c. Turn on the pump and adjust the setting to a flow rate of 5 mL/min during the perfusion.

△ CRITICAL: Sub-step 11, see Figure 1.

KEY RESOURCES TABLE

| REAGENT or RESOURCE                          | SOURCE                        | IDENTIFIER         |
|---------------------------------------------|-------------------------------|--------------------|
| **Experimental models: Organisms/strains**   |                               |                    |
| Mouse, Strain B6J, Male (2–14 months of age)| The Jackson Laboratory       | 000664             |
| **Chemicals, peptides, and recombinant proteins** |                             |                    |
| EGTA                                        | Sigma                         | E3889-100G         |
| Pronase                                      | Sigma                         | P5147              |
| ROCHE Collagenase                           | Sigma                         | 11088882001        |
| ROCHE DNase I                               | Sigma                         | 10104159001        |
| Nycodenz                                    | Accurate Chemical & Scientific Corporation | AN1002424 |
| Sodium Chloride (NaCl)                      | Fisher Scientific             | BP358-10           |
| Potassium Chloride (KCl)                    | Fisher Scientific             | P217-3             |
| Sodium phosphate monobasic monohydrate (NaH2PO4·H2O) | Fisher Chemical             | CAS10049-21-5      |
| Sodium phosphate, dibasic (Na2HPO4)         | Acros Organics                | A0383688           |
| HEPES                                       | Thermo Fisher Scientific      | 15630080           |
| Sodium bicarbonate (NaHCO3)                 | Fisher Scientific             | S233-500           |

(Continued on next page)
### MATERIALS AND EQUIPMENT

**Continued**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| D-(-) Glucose       | Sigma  | 50-99-7    |
| Calcium chloride dihydrate (CaCl2.2H2O) | Aldrich Chemical | 06403HU |
| Magnesium chloride, 6 hydrate (MgCl2.PO4.6H2O) | Macron Chemicals | 0000238270 |
| Magnesium sulfate heptahydrate (MgSO4.7H2O) | Sigma | 230391-500G |
| Potassium phosphate monobasic (KH2PO4) | EM Science | CAS7778-77-0 |
| Grey’s balanced salt solution (GBSS) | Sigma | G9779 |
| 1× DPBS             | Corning | 08121005 |
| eBioscience™ 1x RBC Lysis Buffer | Thermo Fisher Scientific | 00-4333-57 |
| Trypan Blue solution | Corning | 25900046 |
| Isotrurane          | Covetrus | 029405 |
| **Other**           |        |            |
| High fat high sucrose diet | Research Diets | D12451 |
| Chow diet           | Harlan | Teklad Global 18% Protein Rodent Diet 2018 |
| GE Peristaltic Pump P-1 | Neobits | 1811091 |
| Luer Lock Connect   | Baxter | 2C7461 |
| Polyethylene tubing (I.D. 1.57 mm; O.D. 2.08 mm) | INTRAMEDIC, Clay Adams | PE205 |
| Heat lamp           | Braintree Scientific | HL-1 |
| Rephile water purification system | Rephile | RAFFC0250 |
| Stericup® Quick Release PLUS 0.22 µm polyether sulfone (PES), 250 mL | MilliporeSigma | S2GPU02RE |
| Millex-GV Syringe Filter Unit, 0.22 µm, polyvinylidene fluoride (PVDF), 33 mm, gamma sterilized | MilliporeSigma | SLGV033RS |
| Centrifuge® 70 µm Cell Strainer, White, Sterile | Corning | 352350 |
| 50 mL centrifuge tube | Corning | 430829 |
| 15 mL centrifuge tube | Corning | 430791 |
| Eppendorf tube (1.5 mL) | Eppendorf | 022363204 |
| Cell culture dish (60 mm) | Corning | 430196 |
| Clear Boston Round Bottles with Black Phenolic PolyCone Cap | Fisher Scientific | 02-911-785 |
| Insysy Autoguard Shielded IV Winged Catheter, 18G × 1.16” | BD Medical | 381544 |
| Haussers Scientific Bright-Line™ Counting Chamber | Thermo Scientific | 02-671-51B |
| **Critical commercial assays** | | |
| 10× Genomics Chromium Single Cell 3′ Library and Gel Beads Kit (version 3) | 10× Genomics | 1000075 |

### EGTA Stock Buffer

| Reagent                  | Final concentration | Amount |
|--------------------------|---------------------|--------|
| NaCl (136.89 mM)         | 8,000 mg/L          | 8 g    |
| KCl (5.37 mM)            | 400 mg/L            | 400 mg |
| Na2HPO4.H2O (0.64 mM)    | 88.17 mg/L          | 88.17 mg |
| Na2HPO4 (0.85 mM)        | 120.45 mg/L         | 120.45 mg |
| HEPES (9.99 mM)          | 2,380 mg/L          | 2.38 g |
| NaHCO3 (4.17 mM)         | 350 mg/L            | 350 mg |
| EGTA (0.49 mM)           | 190 mg/L            | 190 mg |
| Glucose (4.50 mM)        | 900 mg/L            | 900 mg |
| ddH2O                    | N/A                 | Fill up to 1 L |
| **Total**                | N/A                 | 1 L    |

Store at 4°C up to 3 months.
### Enzyme Stock Buffer

| Reagent                              | Final concentration | Amount |
|--------------------------------------|---------------------|--------|
| NaCl (136.89 mM)                     | 8,000 mg/L          | 8 g    |
| KCl (5.37 mM)                        | 400 mg/L            | 400 mg |
| NaH₂PO₄·H₂O (0.64 mM)                | 88.17 mg/L          | 88.17 mg |
| Na₂HPO₄ (0.85 mM)                    | 120.45 mg/L         | 120.45 mg |
| HEPES (9.99 mM)                      | 2,380 mg/L          | 2.38 g |
| NaHCO₃ (4.17 mM)                     | 350 mg/L            | 350 mg |
| CaCl₂·2H₂O (3.81 mM)                 | 560 mg/L            | 560 mg |
| ddH₂O                                | N/A                 | Fill up to 1 L |
| **Total**                            | N/A                 | 1 L    |

Store at 4°C up to 3 months.

### Grey’s A Stock Buffer

| Reagent                              | Final concentration (mg/L) | Amount |
|--------------------------------------|-----------------------------|--------|
| KCl (4.96 mM)                        | 370                         | 185 mg |
| MgCl₂·PO₄·6H₂O (1.03 mM)             | 210                         | 105 mg |
| MgSO₄·7H₂O (0.28 mM)                 | 70.0                        | 35 mg  |
| Na₂HPO₄ (0.42 mM)                    | 59.6                        | 29.8 mg |
| KH₂PO₄ (0.22 mM)                     | 30.0                        | 15 mg  |
| Glucose (5.50 mM)                    | 991                         | 495.5 mg |
| NaHCO₃ (2.70 mM)                     | 227                         | 113.5 mg |
| CaCl₂·2H₂O (1.53 mM)                 | 225                         | 112.5 mg |
| ddH₂O                                | N/A                         | Fill up to 500 mL |
| **Total**                            | N/A                         | 500 mL |

Store at 4°C up to 3 months.

### Pronase Working Buffer

| Reagent                              | Final concentration | Amount |
|--------------------------------------|---------------------|--------|
| Pronase                              | 0.543 mg/mL         | 19 mg  |
| Enzyme Stock Buffer                  | N/A                 | 35 mL  |
| **Total**                            | N/A                 | 35 mL  |

Store in 39°C water bath 15 min before and during liver perfusion for a maximum of 2 h.

### Collagenase D Working Buffer

| Reagent                              | Final concentration | Amount |
|--------------------------------------|---------------------|--------|
| Collagenase D                        | 0.5 mg/mL           | 20 mg  |
| Enzyme Stock Buffer                  | N/A                 | 40 mL  |
| **Total**                            | N/A                 | 40 mL  |

Store in 39°C water bath 15 min before and during liver perfusion for a maximum of 2 h.

### Liver Dissociation Buffer

| Reagent                              | Final concentration | Amount |
|--------------------------------------|---------------------|--------|
| Pronase                              | 0.6 mg/mL           | 30 mg  |
| Collagenase D                        | 0.5 mg/mL           | 25 mg  |
| Enzyme Stock Buffer                  | N/A                 | 50 mL  |
| **Total**                            | N/A                 | 50 mL  |

Store in 39°C water bath 15 min before and during liver perfusion for a maximum of 2 h.
**STEP-BY-STEP METHOD DETAILS**

**Step one: Liver perfusion**

- **Timing:** 21–25 min

High quality liver perfusion is critical to wash out red blood cells and completely digest the liver so that it is ready for the dissociation into individual cells.

1. Fill the bubble trap with pre-warmed EGTA Working Buffer before liver perfusion.
2. Euthanize mouse using isoflurane in an animal holding jar.
3. Move animal to a flat experimental table and perform laparotomy as soon as the mouse is dead.
4. Expose Internal Vena Cava (IVC) of the mouse.
   a. Cannulate IVC with 18-gauge catheter.

   **Note:** The site of IVC cannulation is above the renal veins and below the liver.

   b. Stabilize the catheter after cannulation to ensure it does not move throughout the perfusion.
   c. Use absorbable tissues to remove extra fluid around the liver. See Methods video S1.

5. Use all of the 35 mL of EGTA Working Buffer to perfuse liver and wash out RBCs. The total time of EGTA perfusion is 5–8 min depending on the mouse diet.

   **Note:** A well-perfused liver looks pale at the end of perfusion. See Methods video S1.

   a. When the liver starts to look engorged by the EGTA Working Buffer (usually 3–5 s), cut the hepatic vein.
   b. Carefully clamp the IVC above the diaphragm immediately after cutting hepatic vein.

   **Note:** Please make sure the catheter is in place without movement.

6. After perfusing the EGTA Working Buffer, take the end of the tubing out and directly put it into the 50-mL centrifuge tube containing pre-warmed Pronase Working Buffer within 3 s. Perfuse the liver with the entire 35 mL Pronase Working Buffer.

   **Note:** Total perfusion time is 8–10 min.

7. Place heat lamp over the liver during all enzyme perfusion steps including Pronase and Collagenase D Working Buffer perfusions.

   **Note:** Make sure the transition among different buffers is smooth and bubble trap remains filled. See Methods video S2.

8. After perfusing the Pronase Working Buffer, perfuse liver with the entire 40 mL of Collagenase D Working Buffer. Total perfusion time is 8–10 min.

   **Note:** In the end, the liver should be soft and well-digested. See Figure 2.

9. Remove the liver and place it into a 60-mm cell culture dish for further liver dissociation.

   **CRITICAL:** Sub-steps 4 and 5, see Methods video S1. Sub-step 7, see Methods video S2. Sub-step 8, see Figure 2.
Step two: Dissociation of liver cells

Timing: 25 min

This step is to dissociate the digested liver to release all liver cell types. The homogenized mixture is a combination of all liver cells and debris. The goal is to have few clumps of liver tissue.

10. Add 5 to 10-mL of preheated Liver Dissociation Buffer into the 60-mm cell culture dish with the liver. Use tweezers to dissociate liver until the mixture becomes largely homogenous.

Note: Total time is 5–8 min. See Methods video S3.

11. Transfer homogenized liver mixture to a clear Boston round bottle with black phenolic polycone cap.

Note: Add the remaining 40 to 45-mL of pre-heated Liver Dissociation Buffer together with 600 μL DNase Stock Solution into the glass bottle. See Methods video S4.

12. Further dissociate homogenized mixture in glass bottle above using gentle rotation by hand swirling in a 39°C water bath (see video) for 20 min. See Methods video S4.

△ CRITICAL: Sub-step 10, see Methods video S3. Sub-steps 11 and 12, see Methods video S4.

Step three: Gradient centrifugation

Timing: 90 min

This step is to wash homogenized liver tissue mixture to remove debris and undigested liver tissue.

13. Transfer homogenized tissue mixture into a 50-mL centrifuge tube by filtering the mixture through a 70-μm cell strainer.
14. Centrifuge the mixture at 580 g at 4°C for 10 min.
15. Remove and discard the top 40 mL without disturbing the bottom pellet. 10 mL of the supernatant and cell pellet will remain in the centrifuge tube.
16. Add 150 μL DNase Stock Solution to the tissue mixture and using a 10 mL pipette and mix.
17. Add 40 mL GBSS buffer to the tissue mixture using a 10 mL pipette and mix.
18. Centrifuge the mixture at 580 g at 4°C for 10 min and repeat steps 15 and 16.
19. Add 16-mL pre-made Nycondez Buffer to the mixture and use GBSS buffer to bring the total volume to 48 mL. Mix well using a 10 mL pipette.
20. Aliquot 12 mL into four 15-mL centrifuge tubes.
21. Carefully add an additional 3-mL GBSS buffer using a 3-mL syringe with a 25-gauge needle to the top of the 12-mL aliquot so that a gradient can form.

**Note:** Make sure not to disturb the gradient layer. See Methods video S5.

22. Centrifuge the 4 centrifuge tubes at 1,380 \( g \) at 4°C for 17 min. Acceleration of centrifuge is set at 9 while deceleration of centrifuge is set at 0. The total centrifugation time is ~37 min.
23. After centrifugation, the bottom pellet is a mixture of all cell types. See Figure 3.

△ CRITICAL: Sub-step 21, see Methods video S5. Sub-step 23, see Figure 3.

**Step four: Liver cells harvest before single cell RNA sequencing**

△ Timing: 20 min

This step is to obtain viable hepatocytes with other liver cell types.

24. Carefully aspirate the supernatant using a 2-mL plastic serological pipette and discard without disrupting the pellet. See Methods video S6.
25. Add 1 mL RBC Lysis Buffer (stored at 4°C) to each of the four 15 mL centrifuge tubes above.
26. Place tubes on ice for 5 min.
27. Add 13 mL pre-chilled PBS stored at 4°C to each 15-mL tube and mix well using a 10 mL pipette. Centrifuge at 400 \( g \) at 4°C for 4 min.
28. Remove the supernatant and re-suspend pellet in 1 mL pre-chilled PBS stored at 4°C and transfer the mixture to a 1.5 mL Eppendorf tube using 1 mL pipette set at 500 \( \mu \)L.
29. Centrifuge cell suspension at 100 g for 3 min at 4°C. Discard the supernatant, and wash pellet using 1 mL chilled PBS and repeat centrifugation to harvest hepatocytes.
**Alternatives:** Centrifuge cell suspension at 400 g for 4 min at 4°C to harvest both hepatocytes and non-parenchymal cells.

30. Resuspend hepatocytes using 500–1,000 μL chilled PBS at 4°C for cell counting and determination of cellular viability by trypan blue staining.

**Note:** If experiment is successful, the hepatocyte viability should be 70%–90%. See Figure 4.

31. The final single cell suspension is used to generate libraries for all liver cells as described in the 10× Genomics Chromium Single Cell 3’ Library and Gel Beads Kit (version 3) manufacturer’s protocol. [https://assets.ctfassets.net/an68im79xiti/51xGuiJhVKOellcW88gsQ/1db2c9b5c9283d183ff4599f4b489a720/CG000183_ChromiumSingleCell3__v3_UG_Rev-A.pdf](https://assets.ctfassets.net/an68im79xiti/51xGuiJhVKOellcW88gsQ/1db2c9b5c9283d183ff4599f4b489a720/CG000183_ChromiumSingleCell3__v3_UG_Rev-A.pdf).

**Alternatives:** 10× Genomics Chromium Single Cell 3’ Library and Gel Beads Kit (version 3) is no longer available due to product upgrade. The equivalent kit can be used. [https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/library-prep/chromium-single-cell-3-reagent-kits-user-guide-v-3-1-chemistry-dual-index](https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/library-prep/chromium-single-cell-3-reagent-kits-user-guide-v-3-1-chemistry-dual-index).

△ CRITICAL: Sub-step 24, see Methods video S6. Sub-step 30, see Figure 4.

**EXPECTED OUTCOMES**

Optimal scRNA-seq data with viable liver cells especially hepatocytes. Please refer to reference 1 Wang et al. (2021) for details.

**LIMITATIONS**

Enzyme amount and corresponding perfusion time (pronase and collagenase concentrations) may need to be optimized for mice fed special diets. There should be no pause or breaks during the entire process. The faster the procedure can be completed, the better the yield of viable hepatocytes. After gradient centrifugation, transfer all the hepatocytes on ice for better viability. This protocol is originally optimized to yield as many viable hepatocytes as possible without using FACS for single cell RNA-seq. For mice fed chow, fewer non-parenchymal liver cells might be obtained. For the mice fed the high fat high sucrose diet, high yield of both hepatocytes and non-parenchymal cells can be obtained. Based on our published data, if centrifuge cell suspension at 400 g for 4 min at 4°C, the percentage of hepatocytes isolated from the 8-week-old male mice fed a chow ranges between 80%–90% and that of non-parenchymal cells ranges between 10%–20% (stellate...
cells 3%–5%; Kupffer cells 3%–4%; endothelial cells 3%–7%, others 1%–4%). In addition, the percentage of hepatocytes isolated from the 26-week-old male mice fed the high fat high sucrose diet for 16 weeks ranges between 60%–80% and that of non-parenchymal cell ranges between 20%–40% (stellate cells 7%–12%; Kupffer cells 6%–10%; endothelial cells 6%–11%, others 1%–7%).

TROUBLESHOOTING

Problem 1
For mice fed other special diets, including but not limited to NAFLD and/or NASH diets, using the indicated concentrations of pronase buffer and collagenase buffers with the same perfusion flow rate for perfusion may not obtain equivalent perfusion results.

Potential solution

- Recommend increasing the pronase working buffer concentration from 0.543 mg/mL to 0.724–0.814 mg/mL and increasing the collagenase working buffer concentration from 0.500 mg/mL to 0.667–0.750 mg/mL depending on the type and duration of special diet feeding.
- Also recommend reducing the perfusion flow rate from 5.5 mL/min to 4 mL/min.

Problem 2
For liver dissociation step, the more homogenous the liver tissue mixture is, the better the outcome. For bigger livers or livers from mice fed a special diet, a homogenous tissue mixture may not be obtained as easily as livers from chow fed mice.

Potential solution

- Recommend using 1/4 to 1/3 of liver for liver dissociation given that only approximately 10k cells are needed for final scRNA-seq sample loading.
- Recommended more vigorous rotation of the glass bottle containing final liver mixture.

Problem 3
Liver cells are very fragile and susceptible to cell death. Poor liver cell scRNA-seq data due to poor liver cell viability (below 70%).

Potential solution

- Recommend no breaks during the entire experimental procedure.
- Recommend having all needed solutions and equipment ready during centrifugation to avoid any unnecessary breaks.
- Recommend transporting centrifuge tubes containing liver cells on ice from the gradient centrifugation step on to reduce time liver cells are at room temperature.
- Recommend immediate proceeding to scRNA-seq sample loading after the last step.

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Simeng Wang (Simeng.Wang@UTSouthwestern.edu).

Materials availability
This study did not generate new unique reagents.

Data and code availability
This study did not generate/analyze data codes.
SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2022.101824.

ACKNOWLEDGMENTS
I thank Dr. Jay D. Horton for manuscript revision and Tessa Edwards for excellent assistance for recording videos of key experiment steps. The work is supported by the NIH HL-20948 and P01HL-160487.

AUTHOR CONTRIBUTIONS
S.W. did all optimization and is the corresponding author.

DECLARATION OF INTERESTS
There are no interests declared.

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