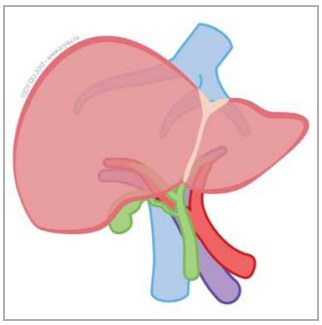

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WORKS FOR ME 1

Human Liver Tissue Storage Methods For Multiomic Applications

COMMENTS 1

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Human Cell Atlas Method Development Community

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ABSTRACT

Sampling different areas of the diseased or healthy liver and storing the tissue in a variety of mediums to allow for an in-depth characterization of heterogeneity and the identification of unique cell populations through the employment of multiomic technologies, such as single-cell RNA-sequencing, single-nucleus RNA-sequencing, flow cytometry, and spatial transcriptomics.

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KEYWORDS

Multiomics, Liver, Tissue Storage, Transcriptomics, human cell atlas methods development community

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GUIDELINES

All biological samples should be handled and processed according to Biosafety Guidelines. This procedure should be performed in accordance with all applicable safety procedures and using aseptic technique.

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MATERIALS TEXT

General Materials:

- 1 x ice bucket
- 2 x sterile scalpels
- 1 x sterile spring scissor (14 mm cutting edge, 0.275 mm tip diameter, 12 cm length, Fine Science Tools)
- 2 sterile forceps
- Flat ice pack
- Petri dishes
- HBSS 1X with Ca^{2+} and Mg^{2+} (Gibco A14025-092, Bleiswijk, The Netherlands)
- 50 ml conical tube

OCT-Embedding Materials:

- 1 x dry-ice bucket
- Pre-labeled base mold (15 x 15 x 5 mm, Ref 22363553, Fisher Brand)
- O.C.T. (Optimal Cutting Temperature) Compound (Fisher Health Care 4585)
- Plastic Film (to wrap the OCT blocks)

Formalin-Fixing Materials:

- Formalin solution, neutral buffered, 10% (Sigma, HT501128)
- 1x 50mL conical tube

Snap-Freezing Materials:

- 1x liquid nitrogen canister
- 1L liquid nitrogen
- 1.5mL cryovials

Sample Collection

1 Collect explanted tissue section in 30mL of HBSS 1x with Ca^{2+} and Mg^{2+} in a 50mL conical tube and keep on ice, ideally to process within 30 minutes of removal from patient or removal from the flushed donor organ in preservation solution.

2 Caudate will be perfused and dissociated as described in Human Liver Caudate Lobe Dissociation for ScRNA-seqV.2

Optimal Cutting Temperature (OCT)-Embedding Tissue Sections

- **For the purpose of spatial transcriptomics**, OCT-embed for the viable freezing and storing for

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3 future slicing of tissue for spatial analyses.
- O.C.T. (Optimal Cutting Temperature) Compound (Fisher Health Care 4585)
- *Additional approach:* Tissue snap frozen in liquid nitrogen can be embedded in OCT after LN storage and then processed for spatial transcriptomics.

3.1 Tissue should remain on ice in HBSS 1X with Ca^{2+} and Mg^{2+} until ready to be sectioned.

3.2 Transfer tissue to a petri dish placed on top of a flat ice pack to begin sectioning with sterile scalpels and sterile forceps.

3.3 Section tissue for OCT-embedding, it should be equal to or smaller than 1cm x 1cm x 0.3 cm (W x L x H).

3.4 Remove excess liquid around the tissue (e.g. by using a dry petri-dish surface).

3.5 Cover the base of the mold with a flat layer of OCT and place the tissue on top (OCT blocks must be pre-labeled).

3.6 Add OCT around and on top of the tissue (the tissue must be completely submerged in OCT).

Note

To ensure complete submersion in OCT, avoid the presence of bubbles above or surrounding the tissue.

3.7 Place the OCT blocks on top of a flat layer dry ice.

3.8 When the block looks completely white, wrap them with plastic microfilm and secure the plastic, make sure to re-label on top of the plastic microfilm.

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3.9 Store the blocks in -80ºC Fridge until future usage.

-80 °C

5m

Formalin-Fixing Paraffin-Embedding

4 **For the purpose of histology and immunohistochemistry,** formalin-fixing and paraffin-embedding (FFPE) tissue allows for the long-term storage of tissue.

4.1 Fill and label a 15mL conical tube with 10mL of **10% Formalin**.

Note

Ensure label contains sample ID, date, lab name, and solution (10% Formalin).

4.2 Ensure to keep the tissue in the petri dish on the ice pack while sectioning, aim for a size of 1cm x 1cm x 1cm (W x L x H).

4.3 Place the tissue section in the tube, fully submerged in 10% Formalin and keep it in the fridge until you are ready to submit it for paraffin-embedding and sectioning for stains.

4.4 **To ensure long-term storage and maintenance of RNA quality, ensure that the FFPE blocks are placed in 4ºC fridge.**

4 °C

15m

Snap-Freezing Tissue Sections

5 **For the purpose of single-nucleus RNA-sequencing** snap-freeze (dry) for the maintenance and storage of nuclei.

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5.1 Fill a canister with 1L of liquid nitrogen.

5.2 Label 1.5ml or 2ml cryovials with study ID, date, lab name, and "Snap-Frozen."

5.3 Section tissue for snap-freezing, it should be approximately 1cm x 1cm x 0.3 cm (W x L x H) in size.

5.4 Remove as much excess liquid as possible by placing it on a dry petri dish surface, then transfer to cryovial, close it shut and drop it in the liquid nitrogen container.

5.5 Once frozen, use forceps to remove them from the container, and place them a cardboard box in a liquid nitrogen storage tank until future usage.

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