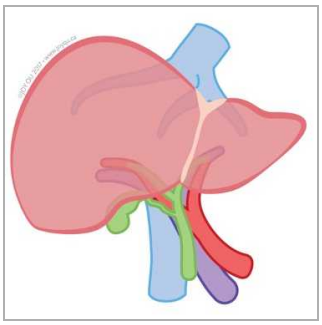

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WORKS FOR ME 1

Human Liver Core-Needle Biopsy Processing Protocol

COMMENTS 0

DOI
dx.doi.org/10.17504/protocols.io.8epv5jzwjl1b/v1

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Human Cell Atlas Method Development Community

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ABSTRACT

Dissociating and isolating cells from a liver biopsy for single cell RNA-sequencing and storage for additional multiomic applications. We have developed a high-thoughput method of dissociating liver tissue for transcriptomic analysis, as well as preparing sections for histological, immunohistochemical and functional assessment.

DOI

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PROTOCOL CITATION

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KEYWORDS

Cell isolation, Core Needle Biopsy, liver, human cell atlas methods development community

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GUIDELINES

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

MATERIALS TEXT

Liver Core-Needle Biopsy Collection and Dissociation:

- 1 x ice bucket
- Pipette Gun
- 5 mL sterile serological pipettes
- P1000 micropipette & suitable sterile tips
- P200 micropipette & suitable sterile tips
- 4 x 50 mL conical tubes
- 1 x 70 µm cell strainer (Falcon® 352350, Durham, DC, USA)
- 1 x sterile spring scissor (14 mm cutting edge, 0.275 mm tip diameter, 12 cm length, Fine Science Tools)
- 1 x 5 mL round-bottom tube (FACS tube)
- 3 x 1.5 mL sterile Eppendorf tube
- 2 x petri dishes
- 1 x polystyrene disposable sterile forceps (RK-06443-20, Cole-Parmer, QC, Canada)
- 1 x Magna 23 disposable scalpels (Almedic)
- PBS 1X (Gibco 10010-023, Paisley, UK) (without Ca2+ and Mg2+)
- BSA (20 mg/mL, New England Biolabs, B9000S, Ipswich, MA, USA)
- FBS (Heat Inactivated, Gibco 12484-028, Grand Island, NY, USA)
- 12 mL HBSS 1X with Ca^{2+} and Mg^{2+} (Gibco A14025-092, Bleiswijk, The Netherlands)
- 1 x aliquot of collagenase (50 µL) (0.5m CDA units/mL, VitaCyte, 001-2030, Indianapolis, IN, USA)
- 1 x aliquot of protease (50 µL) (0.55 NP units/mL, VitaCyte, 003-1000, Indianapolis, IN, USA)
- ACK lysing buffer (Gibco A10492-01, Grand Island, NY, USA)

10m

Buffers and Solutions

1 **Biopsy collection tube:**

Add 10 mL HBSS 1X to a 50 mL tube and keep it on ice or in the fridge for the liver biopsy collection (label the tube with the sample ID and date). All catalogue numbers in materials.

2 **PBS-0.04% BSA:**

Add 100 µL of 2% BSA (same as 20 mg/mL) to 4.9 mL of PBS.

3 **PBS-5% FBS:**

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Add 2.5 mL of FBS to 47.5 mL of PBS.

4 **Enzyme Master Mix:**

Add 50 µL collagenase + 50 µL protease to 0.4 mL of HBSS 1X.

Note

IMPORTANT: *The enzyme mix must be prepared within 30 minutes of use and kept at* 37ºC.

37 °C

Note

- 1 x aliquot of collagenase (50 µL) (0.5m CDA units/mL, VitaCyte, 001-2030, Indianapolis, IN, USA)
- 1 x aliquot of protease (50 µL) (0.55 NP units/mL, VitaCyte, 003-1000, Indianapolis, IN, USA)

10m

Tissue Assessment and Allocation

5 Transfer the biopsy to a petri dish and examine its size and whether it is possible to split it into 2 sections: one half for tissue dissociation and another half to then be split into two or three and be stored as 1) snap-frozen, 2) in OCT and/or 3) in formalin.

Tip: *Place the petri dish on a flat ice pack to section the biopsy while on ice*

6 Using the disposable scalpel and forceps, measure and the biopsy for dissociation and storage according to the below measurements:

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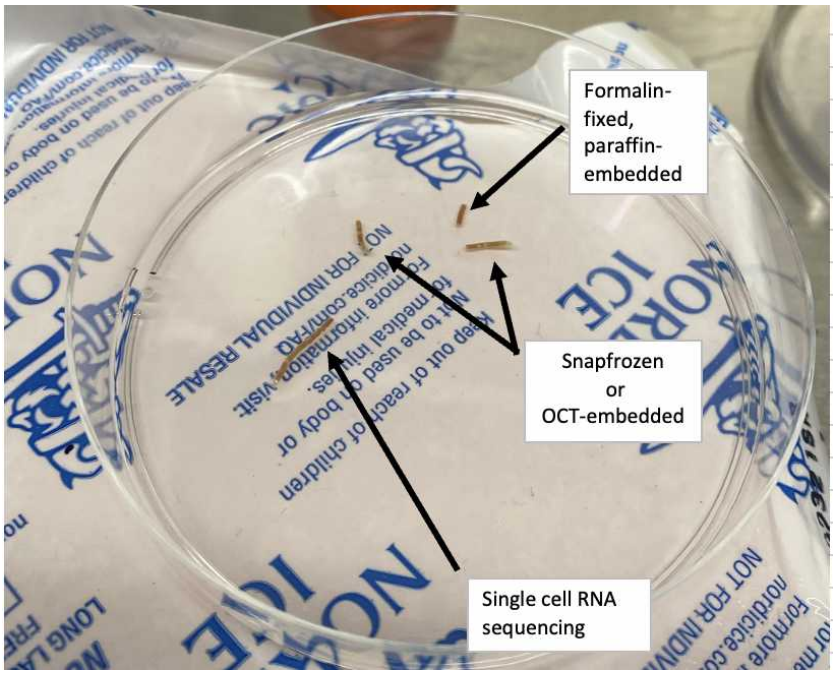
Length of core-needle liver biopsy is 2.4cm, measured with the rule at tail end of the scalpel.



Lengths of core-needle liver biopsy allocated for different analyses and storage methods.

6.1 *For tissue dissociation/scRNA-seq:*

Minimum biopsy length of ~1.0 cm should be allocated.

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Note

Proceed to Step 7 to proceed with the dissociation of the biopsy.

6.2 ***For snap-freezing/OCT-embedding/formalin-fixing:***

Minimum biopsy length of ~0.5 cm for each storage method.

Methods for snap-freezing, OCT-embedding and formalin-fixing are described in Human Liver Tissue Storage Methods For Multiomic Applications protocol.

30m

Biopsy Dissociation

7 Transfer the biopsy section to be dissociated into a 1.5 mL Eppendorf tube containing 200 µL of ***enzyme mix*** kept at 37ºC.

8 Using a sterile spring scissor cut the biopsy into as many pieces as possible. 1 x sterile spring scissor (14 mm cutting edge, 0.275 mm tip diameter, 12 cm length, Fine Science Tools)

9 Secure the Eppendorf tube inside a petri dish and place it in an incubator (37ºC) on a shaker set at 500 rpm for 10 min.

500 rpm, 37°C, 00:10:00

10 **Examine for the presence of undigested fragments.**

If there are undigested fragments, **repeat Steps 8 and 9** to improve biopsy dissociation and digestion.

15m

Filtering and Washing of Cells

11 **Filter the cell homogenate** using a 70 µm cell strainer on a 50 mL conical tube or through the strainer of a 5 mL round-bottom FACS tube.

Tip: *Use a 20-200 µL pipette to transfer the cell suspension to central point of the cell strainer.*

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12 **Wash the cell strainer** with 3ml of **PBS-5% FBS.**

5m

13 **Spin the cell suspension** at 400g for 5min at 4ºC.

400 x g, 4°C, 00:05:00

13.1 Remove the supernatant.

15m

Red Blood Cell Lysis

5m

14 **If the pellet is Pink/Red in color:**

Treat the cells with 1 mL of ACK lysing buffer for 5 minutes at room temperature.

00:05:00

Room temperature

14.1 Add 3ml of **PBS-5% FBS.**

5m

14.2 **Spin cell suspension** at 400g for 5min 4ºC.

400 x g, 4°C, 00:05:00

14.3 Remove supernatant.

5m

Submitting Sample for Single-cell RNA Sequencing

15 Resuspend the pellet in 100ul of **PBS-0.04% BSA.**

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15.1 Transfer the cell suspension to a 1.5ml Eppendorf tube and keep the cells on ice for processing for 10x Genomics scRNA-seq.

On ice

15.2 Left over cells can be counted, spun down with the below specifications, and resuspended in 1ml of Freezing Media (10% DMSO in FBS) and placed in a cryovial for storage.

5m

400 x g, 4°C, 00:05:00

Note

For viable freezing, transfer the cryovial to a Mr. Frosty container in a -80C freezer for 24 hours. Following this, transfer the cryovial to a Liquid Nitrogen Tank for long term storage.

Wrapping up

16 **After completing the protocol,** make sure to clean the scissors and send it for autoclaving (cover the sharp part with a 1.5 mL Eppendorf tube and secure it with an autoclave tape).

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