Sep 30, 2020

Nuclei Isolation from Human Frozen Liver

Sanjay Subramanian[1], Stacey S Huppert[2,3]

[1][Division of Gastroenterology, Hepatology & Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States] ;

[2][Division of Gastroenterology, Hepatology & Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States; [3]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, United States]

1 Works for me dx.doi.org/10.17504/protocols.io.bhpej5je

Sanjay Subramanian

ABSTRACT

This protocol is used to isolate human liver nuclei from frozen samples in an effort to facilitate human nuclei extraction for use in single nuclear RNA sequencing and single cell ATAC sequencing without the need for fresh samples. This approach enables the ability to acquire signatures from all cell types, even those from fibrotic liver samples. A brief fixation step is included in order to preserve nuclear membrane integrity and prevent nuclear leakage. This protocol has been tested and works in frozen human liver samples weighing approximately 1 gram stored in cryopreservation medium. Single nuclear RNA seqencing was run using the 10x Chromium system.

This project was supported in part by NIH P30 DK078392 (Gene Analysis Core) of the Digestive Diseases Research Core Center in Cincinnati, R01DK107553, and the Cincinnati Pediatric Cell Atlas Center funded by Cincinnati Children's Research Foundation to Stacey S. Huppert

DOI

dx.doi.org/10.17504/protocols.io.bhpej5je

PROTOCOL CITATION

Sanjay Subramanian, Stacey S Huppert 2020. Nuclei Isolation from Human Frozen Liver. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bhpej5je

CREATED

Jun 18, 2020

LAST MODIFIED

Sep 30, 2020

PROTOCOL INTEGER ID

38342

GUIDELINES

Sample Storage

Samples are prepared by incubating fresh tissue chunks in HypoThermosol® FRS solution for 15 minutes on ice, followed by a 30 minute incubation in CryoStor® CS10 cryopreservation medium on ice. Samples are then frozen slowly in a cryo-freezing container overnight at -80 °C and subsequently transferred to liquid nitrogen storage.

10x PBS (1L)

80g NaCl

2g KCl

11.5g Na_2HPO_4 $7H_2O$

2g KH_2PO_4
1L diWater

1x PBS with 1% BSA
Make up 100mL 1x PBS by diluting 10mL 10x PBS in 90mL MilliQ water
Make up 100mL 1% BSA in 1x PBS by dissolving 1g BSA in 100mL 1x PBS

4% PFA
10mL of 16% Paraformaldehyde
30mL of 1x PBS

2x Stock ST Buffer (20mL)

Reagent	Volume	Final Concentration
5M NaCl	1.168mL	292mM
1M Tris-HCl pH 7.5	400µL	20mM
1M CaCl2	40µL	2mM
1M MgCl2	840µL	42mM
1x PBS with 1% BSA	17.548mL	
SUPERaseIN	4µL	

1x ST Buffer (5mL)
2.5mL of 2x Stock ST Buffer
2.5mL of 1x PBS with 1% BSA

1x ST Buffer with 0.1% PFA (5mL)
2.5mL of 2x Stock ST Buffer
125µL of 4% PFA
2.374mL of 1x PBS with 1% BSA
1uL of SUPERaseIN

TST Homogenization Solution (5mL)
2.5mL of 2x Stock ST Buffer
120µL of Tween-20
2.375mL of 1x PBS with 1% BSA
5µL of RNase inhibitor

MATERIALS

NAME	CATALOG #	VENDOR
Paraformaldehyde, 16% (wt/vol)	15710	Electron Microscopy Sciences
CryoStor® CS10 100 mL	7930	Stemcell Technologies
Magnesium chloride solution for molecular biology (1.00 M)	M1028	Sigma – Aldrich
Recombinant RNAse Inhibitor	2313A	Takarabio
TWEEN® 20	P7949	Sigma Aldrich
NaCl (5 M) RNase-free	AM9759	Thermo Fisher Scientific
HypoThermosol® FRS Preservation solution	H4416	Sigma Aldrich
Falcon™ Cell Strainers, Mesh size: 70um; white	087712	Thermo Fisher
UltraPure™ 1 M Tris-HCl Buffer, pH 7.5	15567027	Thermo Fisher
SUPERase• In™ RNase Inhibitor (20 U/μL)	AM2696	Thermo Fisher
40um Cell Strainer	22363547	Fisher Scientific
Albumin Bovine Fraction V (BSA)	A30075	Research Products International (rpi)
1 mL Tissue Grinder Dounce	357538	Duran Wheaton Kimble
Calcium chloride 1 M in aqueous solution	97062-820	Vwr

Citation: Sanjay Subramanian, Stacey S Huppert (09/30/2020). Nuclei Isolation from Human Frozen Liver. https://dx.doi.org/10.17504/protocols.io.bhpej5je

NAME	CATALOG #	VENDOR

ABSTRACT

This protocol is used to isolate human liver nuclei from frozen samples in an effort to facilitate human nuclei extraction for use in single nuclear RNA sequencing and single cell ATAC sequencing without the need for fresh samples. This approach enables the ability to acquire signatures from all cell types, even those from fibrotic liver samples. A brief fixation step is included in order to preserve nuclear membrane integrity and prevent nuclear leakage. This protocol has been tested and works in frozen human liver samples weighing approximately 1 gram stored in cryopreservation medium. Single nuclear RNA seqencing was run using the 10x Chromium system.

This project was supported in part by NIH P30 DK078392 (Gene Analysis Core) of the Digestive Diseases Research Core Center in Cincinnati, R01DK107553, and the Cincinnati Pediatric Cell Atlas Center funded by Cincinnati Children's Research Foundation to Stacey S. Huppert

1 Thaw small tissue sample from frozen preservation medium at room temperature and wash in 1x PBS

2 Briefly mince tissue into small chunks using a scalpel and/or razor in petri dish on ice

3 Homogenize chunks using Tissue Grinder Dounce with Pestle A in 500µL to 1mL of ice-cold TST buffer

1 mL TST buffer

1mL Tissue Grinder Dounce comes with two pestles with varying clearance. Pestle "A" is best suited for this protocol

Ensure thorough homogenization, even after solution seems sufficiently aqueous. Quality of fixation depends on achieving full dissociation of cellular membranes

4 Filter suspension through a 70µM cell strainer and bring total volume up to 5mL with ice-cold ST buffer with 0.1% PFA. Ensure solution is mixed well to circulate fix

5 mL ST buffer with 0.1% PFA

5 Centrifuge nuclei at 500g for 5min at 4 °C

00:05:00 centrifuge 500g at 4 °C

6 Remove supernatant and resuspend in 5 mL ST buffer

5 mL ST buffer

7 Repeat steps 5-6

Citation: Sanjay Subramanian, Stacey S Huppert (09/30/2020). Nuclei Isolation from Human Frozen Liver. https://dx.doi.org/10.17504/protocols.io.bhpej5je

Final resuspension volume may be greater or less than stated and should be judged accordingly based on pellet size

8 Filter solution through a 40µM cell strainer

9 Analyze nuclei morphology using hemocytometer and Trypan blue

10 Adjust nuclei concentration as needed (700-1200 cells/µL for 10x Chromium system)

1000 nuclei/µL

Citation: Sanjay Subramanian, Stacey S Huppert (09/30/2020). Nuclei Isolation from Human Frozen Liver. https://dx.doi.org/10.17504/protocols.io.bhpej5je