



Version 3

Sep 15, 2020

Preparation of single cell suspensions from human intestinal biopsies for single cell genomics applications V.3

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In Development

dx.doi.org/10.17504/protocols.io.bmcek2te

Human Cell Atlas Method Development Community

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ABSTRACT

The protocol is adapted from Fujii's and Smillie's reports for single cell transcriptome analysis from human intestines. It provides details on acquirement of single cell suspension from epithelium and lamina propria. This methods is modified to generate appropriate materials from patient's intestinal biopsies for sinlge-cell transcriptome and genomic applications.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

1. Fujii M, Matano M, Toshimitsu K, Takano A, Mikami Y, Nishikori S, Sugimoto S, Sato T. Human Intestinal Organoids Maintain Self-Renewal Capacity and Cellular Diversity in Niche-Inspired Culture Condition. *Cell Stem Cell*. 2018 Dec 6;23(6):787-793.e6. doi: 10.1016/j.stem.2018.11.016. 2.Smillie CS, Biton M, Ordovas-Montanes J, Sullivan KM, Burgin G, Graham DB, Herbst RH, Rogel N, Slyper M, Waldman J, Sud M, Andrews E, Velonias G, Haber AL, Jagadeesh K, Vickovic S, Yao J, Stevens C, Dionne D, Nguyen LT, Villani AC, Hofree M, Creasey EA, Huang H, Rozenblatt-Rosen O, Garber JJ, Khalili H, Desch AN, Daly MJ, Ananthakrishnan AN, Shalek AK, Xavier RJ, Regev A. Intra- and Inter-cellular Rewiring of the Human Colon during Ulcerative Colitis.*Cell*. 2019 Jul 25;178(3):714-730.e22. doi: 10.1016/j.cell.2019.06.029.

DOI

dx.doi.org/10.17504/protocols.io.bmcek2te

PROTOCOL CITATION

Ran RZ Zhou, Oni Basu 2020. Preparation of single cell suspensions from human intestinal biopsies for single cell genomics applications. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bmcek2te>
Version created by [Ran Zhou](#)

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

1. Fujii M, Matano M, Toshimitsu K, Takano A, Mikami Y, Nishikori S, Sugimoto S, Sato T. Human Intestinal Organoids Maintain Self-Renewal Capacity and Cellular Diversity in Niche-Inspired Culture Condition. *Cell Stem Cell*. 2018 Dec 6;23(6):787-793.e6. doi: 10.1016/j.stem.2018.11.016. 2.Smillie CS, Biton M, Ordovas-Montanes J, Sullivan KM, Burgin G, Graham DB, Herbst RH, Rogel N, Slyper M, Waldman J, Sud M, Andrews E, Velonias G, Haber AL, Jagadeesh K, Vickovic S, Yao J, Stevens C, Dionne D, Nguyen LT, Villani AC, Hofree M, Creasey EA, Huang H, Rozenblatt-Rosen O, Garber JJ, Khalili H, Desch AN, Daly MJ, Ananthakrishnan AN, Shalek AK, Xavier RJ, Regev A. Intra- and Inter-cellular Rewiring of the Human Colon during Ulcerative Colitis.*Cell*. 2019 Jul 25;178(3):714-730.e22. doi: 10.1016/j.cell.2019.06.029.

KEYWORDS

single cell, RNA-seq, human intestines

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CREATED

Sep 14, 2020

LAST MODIFIED

Sep 15, 2020

PROTOCOL INTEGER ID

42086

GUIDELINES

The human intestinal tissue are obtained after patients' consents and approval from Institutional Review Board at the University of Chicago (IRB Number: 15573A). All the samples are processed for research use only.

MATERIALS

NAME	CATALOG #	VENDOR
BSA	A7906	Sigma Aldrich
Liberase TM	000000005401119001	Sigma Aldrich
FBS		Invitrogen - Thermo Fisher
Corning® 40µm Cell Strainer	431750	Corning
TrypLE™ Express Enzyme	12604013	Thermo Fisher Scientific
Trypan Blue Solution 0.4% (w/v) in PBS pH 7.5 ± 0.5	25-900-CI	Corning
Eppendorf tubes 1.5 mL uncolored	022363204	Eppendorf Centrifuge
NxGen® RNase Inhibitor	30281-2	Lucigen
Penicillin-Streptomycin (10,000 U/mL)	15140163	Thermo Fisher
DNase I, RNase-free, HC (50 U/µL)	EN0523	Thermo Fisher
HEPES 1M	15630080	Thermo Fisher Scientific
15 ml conical tube	352095	Corning
0.5M EDTA	2482-500	Fisher Scientific
HBSS no calcium no magnesium	14175-095	Thermo Scientific
10 x PBS no calcium no magnesium	BP399500	Fisher Scientific
UltraPure™ DNase/RNase-Free Distilled Water	10977023	ThermoFisher
50 ml Conical tube	28106	Genesee Scientific
0.2 micron syringe filter	7232520	
Filter top vacuum unit 1000 ml	5670020	Thermo Scientific
RBC lysis buffer	11814389001	Sigma Aldrich

MATERIALS TEXT

1x PBS 1000 ml

10x PBS 100 ml

Water 900 ml

Filtered through 1000 ml Filter-top vacuum unit and stored at room temperature.

Wash media 100 ml

HBSS/PBS 98 ml

2% BSA 2 ml

Filtered through 0.2 micron syringe media and prepare freshly

Dissociation media 50 ml

HBSS 47.2 ml

EDTA 0.5M 0.8 ml (final concentration 8 mM)

FBS 1 ml

HEPE 1M 0.5 ml (final concentration 10 mM)

Digestion media 20 ml

Wash media (recipe above) 48.6 ml
Liberase TM (1 mg/ml) 2 ml (final concentration 100 ug/mL)
DNase (5 mg/ml) 0.4 ml (final concentration 100 ug/mL)

Quench media 20 ml

Wash media 19.6 ml
FBS 0.4 ml

Cell suspension buffer 100 ml

PBS 100 ml
2%BSA 0.5 ml

SAFETY WARNINGS

Sharp-end forceps and Iris scissors are used in the protocol. Primary tissues from patients are treated with cautions for unknown infectious status.

DISCLAIMER:

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

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BEFORE STARTING

Generally two samples are obtained from each patient. The sample information from tissue containers (1.7 ml Eppendorf tube in our facility) are recorded. Each sample contains 2-3 pinches. Samples from the same patients but from different biopsy location (ileum and ascending colon) are processed separately. Wet weight of the tissues from each sample are measured by a micro-scale and recorded.

Pre-Dissociation

- 1 Chill wash media and dissociation media on ice. Samples are transferred with Advanced DMEM/F12 based media in 1.7 ml eppendorf tubes on ice. Once received in lab, samples are transferred to 35 mm dish using sharp-end forceps after the media are chilled. Alternatively, tissues can be transferred using a P200 pipette.

On ice



Pinched tissues range from 6 to 20 mm³ in size.

Sample Wash before dissociation

- 2 Place 3 ml ice cold wash media in each dish with each sample (containing 2-3 pinches). Shake the dish on ice to rinse off mucus.
- 3 Repeat step 2 three more times or until the wash media becomes clear.

- 4 Transfer tissues from the 35 mm dish to a clean 5 ml conical tube. Add 0.2 ml Dissociation media and mince the tissues with Iris scissors for 1 minute on ice.



The minced tissues range from 1 to 3 mm³ in size, can easily pass a P1000 tip.

🕒 00:01:00 🧊 On ice

Dissociate Epithelial layers from Lamina propria

- 5 Add 3 ml ice-cold Dissociation media to each 5 ml tube.
- 6 place the tubes on ice on a linear rocker and rock the tubes (end to end) at 100/min for 60 mins. Alternatively rotate the tubes in a 37 Celsius oven for 15 mins.
🧊 On ice 🌡 4 °C 🕒 01:00:00
- 7 Pipet the tissue in dissociation media in a 5 ml serological pipet 5 times and let the tissue settle in the tube on ice for 5 mins. Collect the supernatant and continue with Epithelium Digestion. Add 10 ml dissociation media to the tissue and repeat step 6.
- 8 Collected supernatant will be processed in the following section. Sedimented tissue represents the lamina propria and will be processed in the "Lamina propria Digestion" section.



The epithelial sheets isolated from lamina propria remain in the Dissociation media supernatant. The lamina propria will be processed separately from epithelium.

Epithelium Digestion

- 9 Centrifuge the epithelium in the supernatant in 5 ml conical tubes at 300 g x 5 mins at 4 celsius.
🌀 300 x g, 4°C, 00:05:00
- 10 Remove supernatant and add 2 ml warmed TrypLE express to the pellet. Incubate the mixture at 37 celsius for 5 mins with rotation at 20 rpm.



A hybridation oven with a rotisserie is recommended.

- 11 Neutralize TrypLE express by adding 2 ml wash media, agitate the tissue by pipetting up and down and filter through a 40 micron cell strainer.

- 12 Centrifuge at 300 g x 5 mins at 4 celsius. Remove supernatant. Resuspend cells in 1 ml wash media and place on ice.

 **003 x g, 4°C, 00:05:00**

- 13 Take 20 ul cell suspension with 20 ul Trypan blue and mix well. Check the cell viability on a light microscope. If viability is higher than 85%, proceed with red blood cell removal.



Either hemocytometer or Countess automated cell counter can be used for viability assessment. Live cells are excluded from staining blue and the numbers of live and dead cells are both counted for viability rate = $100\% \times N \text{ of live cells} / (N \text{ of live cells} + N \text{ dead cells})$. At least 50 cells are counted in total.

Lamina propria Digestion

- 14 Wash the tissue one times with 5 ml Wash media. Aspire the Wash media as much as possible.

- 15 Add 3 ml Digest media. Incubate the tissue at 37°C with end-over-end rotation 20 rpm for up to 30 mins.



Check the digestion after 20 mins incubation with Digest media. If cell clumps remains and cell viability > 95%, incubate for additional 5 minutes.

- 16 Neutralize TrypLE by 0.5 ml Quench media. Leave tissue on ice for 5 mins. Agitate the tissue by pipetting up and down and filter through a 40 micron cell strainer.

- 17 Centrifuge at 300 g x 5 mins at 4 Celsius. Remove supernatant and suspend cells in 1 ml wash media.

 **300 x g, 4°C, 00:05:00**

- 18 Take 20 ul cell suspension with 20 ul Trypan Blue. Mix well and check the viability on a light microscope. Proceed with red blood cell removal.

Red blood cell removal

- 19 Dilute one volume of cell suspension (epithelium or lamina propria; keep tissue types separate) by two volumes of Red Blood Cell Lysis Solution.

- 20 Gently mix by inverting the tubes and incubate for 2 minutes at room temperature.

- 21 Centrifuge at 300 g for 5 minutes at 4 Celsius.

 **300 x g, 4°C, 00:05:00**

- 22 Aspirate supernatant completely. Resuspend the cell pellet in 0.1 ml cell suspension buffer with RNase Inhibitor 0.1 U/ul. Proceed with Cell suspension preparation.

Cell Suspension Preparation

- 23 Count the cells with Trypan Blue staining.
- 24 Adjust cell density by cell suspension buffer with RNase Inhibitor 0.1 U/ul.