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

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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 method is modified to generate appropriate materials from patient's intestinal biopsies for single-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.

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	00000005401119001	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

NAME ▾	CATALOG # ▾	VENDOR ▾
HEPES 1M	15630080	Thermo Fisher Scientific
15 ml coriocal tube	352095	Corning
0.5M EDTA	2482-500	Fisher Scientific
HBSS no calsium no magnesium	14175-095	Thermo Scientific
10 x PBS no calsium no magnesium	BP399500	Fisher Scientific
UltraPure™ DNase/RNase-Free Distilled Water	10977023	Thermofisher
50 ml Conical tube	28106	Genesee Scientific
0.2 micron syring filter	7232520	
Filter top vaccum unit 1000 ml	5670020	Thermo Scientific
Red blood cell lysis buffer 10x	130-094-183	Miltenyi Biotec

MATERIALS TEXT

1x PBS 1000 ml

10x PBS 100 ml

Water 900 ml

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

Wash media 100 ml

HBSS/PBS 97 ml

FBS 2 ml

Pen/Strep 1 ml (final concentration 100 units/mL of penicillin and 100 µg/mL of streptomycin)

Filtered through 0.2 micron syring 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

Pen/Strep 0.5 ml (final concentration 100 units/mL of penicillin and 100 µg/mL of streptomycin)

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

1x RBC lysis buffer 50 ml

10x RBC lysis buffer 5 ml

Water 45 ml

Cell suspension buffer 100 ml

PBS 100 ml

BSA 10 mg

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.

BEFORE STARTING

Generally two samples are obtained from each patient. The sample information from tissue containers (1.5 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 medium in 1.5 ml eppendorf tubes on ice. Once received in lab, sample are transferred to 15 ml conical tube using sharp-end forceps after the media are chilled. Alternatively, tissues can be transferred using a P1000 pipette.

 **On ice**



Pinched tissues range from 6 to 20 mm³ in size.

Sample Wash before dissociation

- 2 Place 10 ml ice cold wash media in each 15 ml conical tube with each sample (containing 2-3 pinches). Invert tubes 5 times to rinse off mucus.
- 3 Repeat step 2 four more times or until the wash media becomes clear.
- 4 Transfer tissues from 15 ml conical tube to a clean 1.5 ml Eppendorf tube and mince the tissues with Iris scissors for 2-3 mins on ice.



The minced tissues range from 1 to 3 mm³ in size.

 **00:03:00**  **On ice**

Dissociate Epithelial layers from Lamina propria

- 5 Add 1 ml ice-cold Dissociation media to each eppendorf tube and transfer the mixture to 15 ml conical tubes. Place 9 ml ice-cold Dissociation media in each conical tube to agitate the tissues settled in the bottom.
- 6 Rotate the tubes (end over end) on a rotator in a cold room at 15 rpm for 30 mins. Alternatively place the tubes on ice on a linear rocker and rock the tubes (end to end) at 100/min for 30 mins.

 **On ice**  **4 °C**  **00:30:00**

- 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 1-2 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 15 ml conical tubes at 300 g x 3 mins at 4 celsius.
 300 x g, 4°C 00:03:00
- 10 Remove supernatant and add 5 ml TrypLE express to the pellet. Incubate the mixture at 37 celsius for 10 mins with rotation at 20 rpm.
- 11 Neutralize TrypLE express by adding 5 ml wash media. Centrifuge at 400 g x 3 mins at 4 celsius.
 400 x g, 4°C 00:03:00
- 12 Remove supernatant. Resuspend cells in 1 ml wash media and place on ice.
- 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 three times with 5 ml Wash media. Transfer the tissue with P1000 pipette to 1.5 ml Eppendorf tubes.
- 15 Add 1 ml Digest media. Place tissue at 37°C with 300 rpm on the thermomixer for up to 20 mins.




Check the digestion after 15 mins incubation with Digest media. If cell clumps remains and cell viability > 85%, 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.
- 17 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

- 18 Dilute one volume of cell suspension (epithelium or lamina propria; keep tissue types separate) by ten volumes of Miltenyi Biotec 1× Red Blood Cell Lysis Solution.
- 19 Gently mix by 5 ml serological pipet for 5 seconds and incubate for 2 minutes at room temperature.
- 20 Centrifuge at 400×g for 10 minutes at room temperature.
 **400 x g, Room temperature 00:10:00**
- 21 Aspirate supernatant completely. Resuspend the cell pellet in 5 ml cell suspension buffer and filter through a 40 micron cell strainer. Proceed with Cell suspension preparation.

Cell Suspension Preparation

- 22 Pellet the cells by centrifugation 400 g x 3 mins at 4 celsius.
 **400 x g, 4°C 00:03:00**
- 23 Remove supernatant. Resuspend cells in 1 ml cell suspension with RNase Inhibitor 0.1 U/ul. Check the viability with Trypan blue again. Place the cells on ice for downstream applications.



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