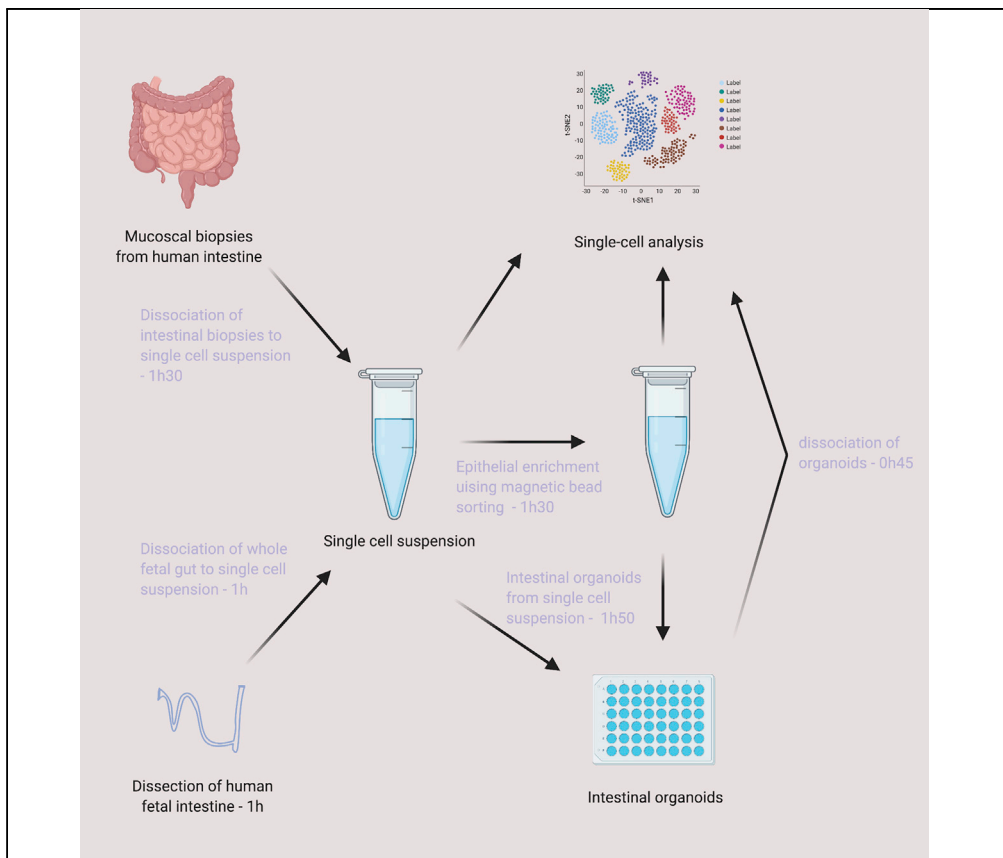


Protocol

Obtaining purified human intestinal epithelia for single-cell analysis and organoid culture



Here, we describe protocols for the preparation and dissociation of human fetal and pediatric intestinal tissue to a high-viability epithelial single-cell suspension. This epithelium-enriched single-cell suspension can then be used to generate single-cell RNA sequencing data as well as to create human intestinal organoids from both the fetal and pediatric intestine. Finally, this protocol details the dissociation of the intestinal organoids for use in single-cell analysis or passaging of organoids.

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Highlights

Dissection of the human embryonic/fetal intestine

Dissociation and enrichment of human intestinal epithelia from fetal/mucosal biopsies

Establishment and maintenance of intestinal organoids from single-cell suspension

Dissociation of organoids to single-cell solution

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Protocol

Obtaining purified human intestinal epithelia for single-cell analysis and organoid culture

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SUMMARY

Here, we describe protocols for the preparation and dissociation of human fetal and pediatric intestinal tissue to a high-viability epithelial single-cell suspension. This epithelium-enriched single-cell suspension can then be used to generate single-cell RNA sequencing data as well as to create human intestinal organoids from both the fetal and pediatric intestine. Finally, this protocol details the dissociation of the intestinal organoids for use in single-cell analysis or passaging of organoids.

For complete details on the use and execution of this protocol, please refer to Elmentaite et al. (2020).

BEFORE YOU BEGIN

△ CRITICAL: pre-coat all plasticware with a 0.1% solution of BSA in PBS prior to use

Preparation for tissue processing to single-cell solution (fetal and pediatric tissue)

⌚ Timing: 15 min

1. Prepare 1.5 mL tubes with 500 μ L of sterile PBS. Prepare a separate tube for each specimen to be collected. Keep the tubes on ice.
2. Prepare a six well plate with each well containing a solution of 1.69 mL of HBSS, 1.07 Wunsch units/mL of Liberase DH (Sigma-Aldrich) and 600 IU of Hyaluronidase (Merck). Place the plate in a standard incubator to ensure it reaches 37°C.
3. Keep sterile DMEM/F-12 (Dulbecco's Modified Eagle Medium) on ice in a 50 mL cylindrical tube.



Preparation for generation of organoids

⌚ Timing: 1 h

4. Remove Matrigel from freezer and thaw on ice.
5. Prepare organoid media according to “Materials and equipment” below. Filter before use with a 33 mm diameter 0.2 µm filter (Fisher). The medium can be stored at 4°C, but ideally not for longer than one week. Additional information on human intestinal organoid culture can be found in the following publications: (Fordham et al., 2013; Kraiczy et al., 2019; Sato et al., 2011).
6. Place sterile 48-well plate into incubator (37°C, 5% CO₂).

Preparation for the dissociation of organoids

⌚ Timing: 15 min

7. Ensure adequate volume (200 µL per sample) of Cell Recovery Solution at 4°C.
8. Place an aliquot (500 µL per sample) of trypLE at 37°C.
9. Place an aliquot of DMEM on ice (3 mL per sample).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Matrigel® (Growth factor reduced, phenol free)	Corning	356231
HBSS (no calcium, no magnesium)	Life Technologies	14170088
Hibemate™ Medium E	Gibco	A1247601
Nicotinamide	Sigma-Aldrich	N3376
N-Acetyl-L-cysteine	Sigma-Aldrich	A7250
Primocin	InvivoGen	ant-pm-2
WNT3A	Conditioned cell line (internal)	N/A
R-Spondin-1	Conditioned cell line (internal)	N/A
Murine EGF	Invitrogen	PMG8041
A83-01	Tocris	2939
SB 202190	Sigma-Aldrich	S7067
Y27632	Selleckchem	S1049
Murine Noggin	PeproTech	250-38
B27	Thermo Fisher Scientific	17504044
HEPES buffer	Gibco (Life Technologies)	15630080
GlutaMAX	Gibco (Life Technologies)	35050061
DMEM/F-12	Gibco (Life Technologies)	11320033
Liberase DH	Sigma-Aldrich	5401054001
WNT2B	Abcam	ab132538
Hyaluronidase	Merck	HX0514
Cell Recovery Solution®	Corning	354253
TrypLE™ Express Enzyme (1 ×), no phenol red	Thermo Fisher	12604021
CD326/EpCAM magnetic microbeads	Miltenyi Biotec	130-061-101
FcR Blocking Reagent	Miltenyi Biotec	130-059-901
Bovine Serum Albumin Standard Ampules	Thermo Fisher Scientific	23209
Fisherbrand™ Sterile PES Syringe Filter	Fisher Scientific	15206869

MATERIALS AND EQUIPMENT

Organoid medium concentration		
Basal Organoid medium = ADF+++	Final concentration	Amount
Products from Gibco by Thermo Fisher Scientific		
Advanced DMEM/F-12 (ADF)	1×	500ml
GlutaMAX	2 mM	5ml
HEPES buffer	10 mM	5ml
Penicillin/Streptomycin	0.5 U/mL	5ml
Complete medium		
	Final concentration	
ADF+++ (see above)	27% (vol/vol)	13.2ml
WNT3A conditioned medium	50 % (vol/vol)	25ml
R-Spondin-1 conditioned medium	20 % (vol/vol)	10ml
Primocin (Invivogen, San Diego, CA, USA)	500 µg/mL	100µL
B-27® Supplement 50× (Invitrogen, Carlsbad, CA, USA)	1×	1ml
Nicotinamide (Sigma, St. Louis, MO, USA)	10 mM	500µL
N-Acetylcysteine (Sigma, St. Louis, MO, USA)	1.25 mM	125µL
A-83-01 (Tocris, Bristol, UK)	500 nM	5µL
SB202190 (Sigma, St. Louis, MO, USA)	10 µM	5µL
Murine EGF (Invitrogen, Carlsbad, CA, USA)	50 ng/mL	25µL
Murine Noggin (PeproTech, Rocky Hill, NJ, USA)	100 ng/mL	50µL

Modified MACS buffer		
Reagent	Final concentration	Amount
PBS	NA	1ml
10% BSA	0.5%	50µL
0.5 M EDTA	2mM	4µL
1000 IU DNase/mL	100µL/mL	100µL

MACS running buffer		
Reagent	Final concentration	Amount
PBS	NA	1L
10% BSA	0.5%	50ml
0.5 M EDTA	2mM	4ml

Alternatives: Recombinant Wnt and R-spondin can be used with similar efficacy. Murine or human EGF may be used.

STEP-BY-STEP METHOD DETAILS

Dissociation of human pediatric mucosal biopsies

⌚ Timing: 2 h

This step processes human intestinal biopsy samples to a single cell solution.

⚠ **CRITICAL:** Mucosal human biopsies should be obtained from individuals after full consent has been given and ethical approval has been obtained for its collection and use for research including for single cell studies.

1. Once obtained, mucosal biopsies should be transferred into a 1.5 mL tube with 500 µL of PBS for short term storage on ice. At least two biopsies (punch mucosal biopsies obtained during an

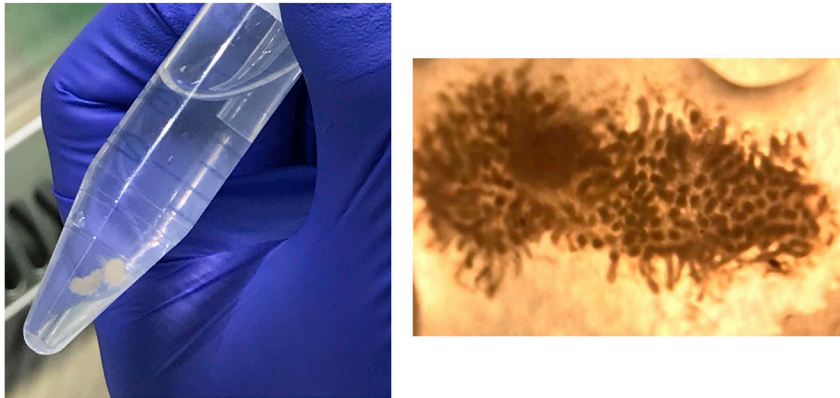


Figure 1. Human pediatric mucosal biopsies in a falcon tube (on the left) and visualized using a bright-field microscope (on the right)

endoscopic procedure) should be used for each digestion, to obtain cell number required for single cell RNA sequencing or organoid culture (Figure 1).

△ CRITICAL: mucosal biopsies vary in their size and depth and therefore cell yield may vary substantially. In general, the maximum amount of tissue available should be used, and the original biopsy size should be considered when later assessing the number of cells recovered.

2. Rinse sample twice with PBS by removing the PBS with a pipette, and then replacing it with fresh PBS.
3. Transfer sample to pre-prepared six well plate (Figure 2). Each sample should be placed in a separate well containing a total volume of 1.69 mL; HBSS (1.3 mL) with 1.07 Wunsch units/mL of Liberase DH (350 μ L) (Sigma-Aldrich) and 600 IU of Hyaluronidase (40 μ L) (Merck).
4. Place the six-well plate on a shaker (Figure 2) and incubate at 37°C for 15 min, rotating at 750 rpm.
5. Once 15 min is complete, remove six-well plate from the incubator and mechanically disrupt the partially digested sample using a pre-coated p1000 pipette. Mechanical disruption involves pipetting the entire mixture up and down several times, during which the structure of the biopsy will visibly reduce, but not disappear entirely.
6. Place the six-well plate back into the incubator and rotate at 750 rpm in 37°C for further 15 min.
7. Remove the sample from incubator and again mechanically disrupt the sample by pipetting up and down until the biopsy structure has been lost and the cells are in suspension; this should be achieved after pipetting for approximately 10 times (Figure 3). At the end of the second mechanical disruption, the sample should almost entirely have broken down and lost its mucosal structure; if this is not the case, then further mechanical disruption with pipetting should be performed.
8. Transfer each sample to a 15 mL conical tube and centrifuge for 5 min at 400 g (Figure 4).
9. Remove the supernatant and dilute with 500 μ L of DMEM/F-12, transfer to 1.5 mL tube, then centrifuge again for 5 min at 400 g (Figure 4).
 - a. Remove the supernatant and repeat this step for a third time
10. Resuspend the sample in 1000 μ L of DMEM/F-12 and filter through a 40 μ m filter to remove any debris or cell clumps (Figure 5). Transfer the filtered sample to a 1.5 mL tube and store on ice until required.
11. To enrich the sample for epithelial cells, the cells can be sorted using EPCAM magnetic beads (see step 31 below).



Figure 2. Six-well plate with digestion solution on a platform shaker

△ **CRITICAL:** If utilising the sample for single cell RNA-sequencing, filter the sample immediately before loading the cells into the machine utilized for single cell assessment (for example, Chromium controller, 10x genomics). The cells may begin to clump after filtration and therefore the shortest time period possible should be maintained prior to loading the sample, if the cells do begin to clump, there is a high chance of the machine becoming clogged and the experiment failing.

Dissection and dissociation of the human fetal intestine

⌚ **Timing:** 2 h

This step describes the steps required to remove the intestine from a fetal sample, and to dissociate this to a single cell suspension. The dissection may be performed at room temperature, with the sample kept at 4°C when not being dissected.

△ **CRITICAL:** Human fetal material should be used only after full regulatory and ethical approval (according to national and local guidelines/regulations) has been obtained for its collection and use for research including for single cell studies.

12. Sample should be collected into a standard sterile petri dish containing 6 mL of Hibernate medium E (A1247601)
13. In a cell culture hood (containment category dependent on sample source) with a microscope, (for example a Leica MZ 75) the sample should be positioned supine. Take two forceps and grip the abdominal wall near to the umbilicus, then gently pull the wall in opposite directions away from the midline. The abdominal wall should readily tear; pull until the abdominal contents are exposed.
14. Remove the liver. This is performed by inserting forceps behind the superolateral aspect of the liver and gently everting the liver from the abdominal cavity. At this point the liver will still be connected to the gut by the biliary system, so to remove the liver cut the biliary tree as close to the duodenum as possible. The liver and biliary tree should now be separate from the abdomen and can be removed.
15. At this stage, the intestine should be intact. Identify the stomach and colon by anatomical location (stomach is cranial, colon is caudal). Identify the stomach and cut the stomach away from the esophagus. Now carefully and slowly remove the stomach from the abdominal cavity into the dish, ensuring that it remains connected to the duodenum.

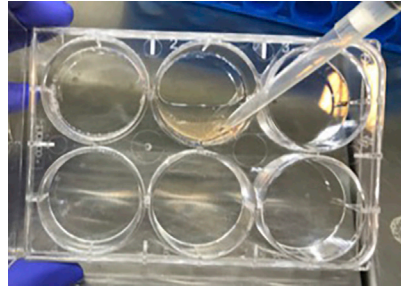


Figure 3. Pipetting mixture in six-well plate to mechanically disrupt the sample

△ **CRITICAL:** Where possible, avoid gripping the intestine with the forceps and use the stomach to mobilize the intestine.

16. The intestine should be able to be removed into the dish in its entirety, however some areas may need dissecting away from connective tissue/mesentery. This should be done by carefully pulling the mesentery away from the intestine, and not directly handling the intestine.
17. At this point, the intestine can be separated entirely from the sample by cutting the distal colon
18. With the intestine now entirely dissected out of the abdominal cavity, carefully remove the remaining connective tissue/mesentery that encases it. Do so by grasping two parts of the same section and pull in opposite directions gently. With light pressure the connective tissue will gradually separate from the intestine. Repeat until the intestine forms a single long tube.
19. Now the stomach can be removed by cutting at the gastroduodenal junction.
20. The colon can be separated by isolating the ileocecal junction and cutting at this site.
21. The small intestine can then be further cut to separate the duodenum/jejunum/ileum
22. Dissected pieces of gut should be transferred to 0.5 mL of PBS (phosphate buffered saline) and kept on ice until further processing.
23. Rinse twice with Hank's Balanced Salt Solution (HBSS) medium (Sigma-Aldrich) by replacing the PBS with 1 mL of HBSS, taking care not to remove any tissue and keeping sample on ice.
24. To digest the tissue, place the sample in HBSS medium containing 1.07 Wunsch units/mL of Liberase DH (Roche) and 600 IU of Hyaluronidase (Calbiochem) in a six-well plate (Figure 2).
25. Place the plate on a shaking platform (750 rpm) at 37°C for 15 min.
26. Mechanically disrupt the digested sample by pipetting the sample up and down using a P1000 pipette. The sample should readily separate to a cellular solution after pipetting 3–4 times, however if this is not the case then pipette further until a cellular solution is achieved.
27. Transfer each sample to a 15 mL conical tube and centrifuge for 5 min at 400 g.
28. Remove the supernatant and dilute with 500 µL of DMEM (Dulbecco's Modified Eagle Medium), transfer to 1.5 mL tube, then centrifuge again for 5 min at 400 g.
 - a. Remove the supernatant and repeat this step for a third time
29. Resuspend the sample in 1000 µL of DMEM and filter through a 40 µm filter to remove any debris or cell clusters. Transfer the filtered sample to a 1.5 mL tube and store on ice until required.
30. To enrich the sample for epithelial cells, the sample can be sorted using EPCAM magnetic beads (see step 31 below).

Enrichment for intestinal epithelial cells

⌚ **Timing:** 1.5 h

31. Centrifuge the single cell suspension that was obtained from either the fetal intestine (step 30) or mucosal biopsy (step 11) for 5 min at 400 g.
32. Remove the supernatant and resuspend the pellet with 300 µL of MACS modified buffer (see materials and equipment).

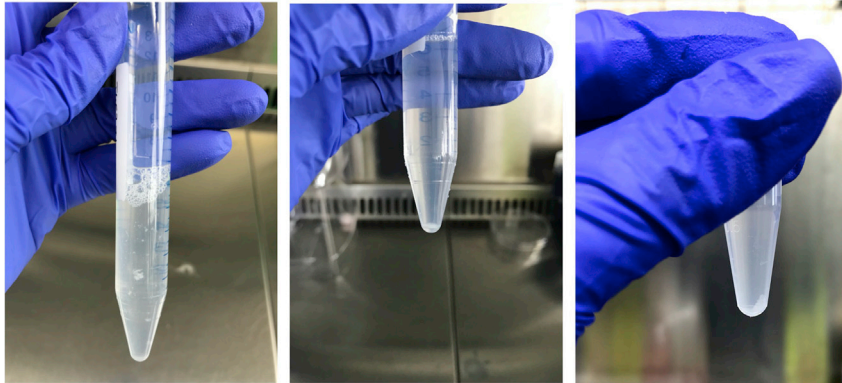


Figure 4. Supernatant in a 15-mL conical tube containing the crypts isolated from biopsies (on the left), crypts pelleted down after centrifugation (in the middle and on the right)

33. Add 100 μ L FcR Blocking Reagent and 100 μ L of CD326 (EpCAM) Microbeads per tube and mix well by pipetting. In general, the cell yield will be less than 1×10^7 , however if the cell number is greater than 1×10^7 then the reagent can be scaled accordingly.
34. Place sample at 4°C for 30 min.
35. Add 5 mL running buffer (see materials and equipment), mix by pipetting, and then centrifuge at 400 g for 5 min.
36. Remove the supernatant and resuspend the pellet in 600 μ L of running buffer then load onto autoMACS machine and perform a “positive selection” automated cellular purification cycle.
37. Centrifuge positively selected sample for 5 min at 400 g and resuspend sample in 500 μ L of DMEM/F-12 and transfer to a 1.5 mL tube. Keep the sample on ice until further processing for single cell analysis or establishment of organoids. Over time the cells will begin to attach to each other, and therefore proceeding as quickly as possible with subsequent processing is advised, as well as passing the suspension through a 40 μ m filter immediately prior to use.

Establishment and maintenance of organoids

⌚ Timing: 1.5 h

38. Centrifuge the single cell suspension obtained from either step 11, 30 or 37, for 5 min at 400 g.
 39. Remove the supernatant and resuspend pellet in liquid Matrigel (calculate total volume of Matrigel as 20 μ L per well, plus 10%). The Matrigel should be fully thawed and kept on ice.
 40. Carefully mix the Matrigel-cell solution to ensure even distribution of the cells throughout the gel. Precise calculation of the number of cells per well is not necessary, however as an approximate guide 5,000 cells per well can be seeded. Aim to seed at least double this number when using unsorted cells. Matrigel can be used in its undiluted form, or diluted with medium up to 20% by volume if desired. Ensure the Matrigel is fully thawed and mixed prior to use (i.e., that it has become liquid, clear, and absent of crystals).
- ⚠ CRITICAL: Maintain the gel and cells on ice as much as possible and avoid prolonged periods of pipetting. If the gel becomes too warm it will begin to set and disrupt pipetting.**
41. Once the cell suspension has fully mixed with the gel, pipette onto a pre-warmed (allow at least 30 min in the incubator for warming) 48 well plate using 20 μ L per well, ensuring the gel does not touch the side of the well (Figure 6). A useful tip for pipetting into a 48 well plate is to position the plate at a 45° angle from the line of site, as this allows visualization of the base of each well and therefore accurate pipetting to the central point.



Figure 5. Setup for filtering single-cell solution

△ **CRITICAL:** Ensure that the Matrigel/cell mix is kept on ice as much as possible; if the gel becomes too warm it will solidify and no longer able to be pipetted. The plate may cool during this process however the gel will still solidify once the plate is placed in the incubator. If the plate has cooled significantly, be aware that the gel will remain more liquid and therefore extra care should be taken when handling the plate for the gel not to touch the sides of the well.

42. Once all the samples are pipetted into the plate, the plate should be inverted and placed in the incubator for five min (Figure 7).

△ **CRITICAL:** Inversion of the plate should be performed as quickly as possible. If the plate is not inverted quickly the liquid gel will run to the edge of the well.

43. Once the Matrigel has set (approximately five minutes at 37°C), pipette 200–250 μ L of intestinal organoid medium (see above) onto each well.

44. Change medium 24 h later, and subsequently every 48 h. To change the medium, tilt the plate to 45°, then use a pipette to remove the medium from the bottom edge of the well, taking care not to disturb/ disrupt the Matrigel dome.

45. To add fresh medium, use medium that has been pre-warmed to 37°C. The medium should be applied to the bottom of the well slowly, so as not to interrupt the Matrigel dome.

46. Organoids can then be passaged either every 5–10 days, either mechanically or via enzymatic digestion (see steps 47 or 56).

△ **CRITICAL:** Rarely, infection may occur which appears as a clouding/opacification of the medium. When this occurs, the unaffected wells should be re-seeded immediately to a new plate, and the affected wells discarded in an appropriate manner.

47. To mechanically passage the organoids, using a p1000 pipette, scrape the Matrigel dome off the surface of the plate and pipette up the gel together with the medium into a 1.5 mL tube. Multiple wells can be split together, with larger plasticware required if more than six wells are used.

48. Gently press the tip of the p1000 pipette into the bottom of the 1.5 mL tube to flatten the aperture of the tip, and then pipette the gel/media mix several times to break up the organoids. Pipetting approximately ten times should dissociate the organoids to fragments that will quickly regenerate complete organoids, however further pipetting will generate ever smaller fragments, if desired. Assessment of this process can be performed by assessing the size of the fragments/organoids under the microscope.

49. Centrifuge mixture at 400 g for 5 min.

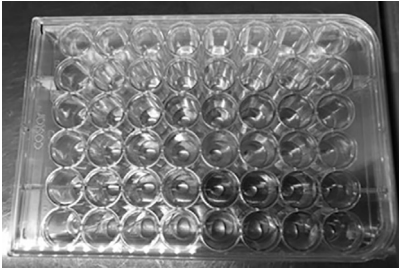


Figure 6. Matrigel domes pipetted into a 48-well plate

50. Remove the supernatant and resuspend the pellet in the desired volume of gel (in general, split at a ratio of 1:6 wells, and total gel volume as 20 μ L per well plus 10%). If desired, other plates can be used, for example a 24-well plate, with the volumes of gel and medium adjusted accordingly, e.g., 40 μ L of gel and 500 μ L medium for a 24 well plate.
51. Pipette 20 μ L of gel to each well of a pre-warmed 48 well plate.
52. Invert the plate and place in the incubator for five minutes (Figure 7).

△ CRITICAL: Inversion of the plate should be performed as quickly as possible. If the plate is inverted quickly the liquid gel will run to the edge of the well.

53. Once the Matrigel has set (approximately five minutes at 37°C), pipette 200–250 μ L of pre-warmed (37°C) intestinal organoid medium (see above) onto each well (Figure 8)
54. Change medium 24 h later, and subsequently every 48 h.
55. Organoid can be maintained in this manner for over one year, and if required can be cryopreserved. To cryopreserve organoids, remove the media from four wells and replace with 1 mL of cell recovery solution (250 μ L per well). Transfer the gel and cell recovery solution to a 1.5 mL tube and keep on ice for 20 min.
56. Centrifuge at 400 g for five min. Remove the supernatant and wash in basal medium twice.
57. Re-suspend the pellet in 500 μ L freezing medium and place in a freezing container, then transfer to a -80° C freezer. After 48–72 h, the sample may then be transferred to storage in liquid nitrogen.
58. To thaw the organoids, place the vial into a water bath at 37°C. As soon as the sample has thawed, wash three times in basal medium, then resuspend in Matrigel and re-plate the organoids with an appropriate volume of medium.

Dissociation of organoids and processing for single-cell RNA sequencing

⌚ Timing: 45 min

This step dissociates intestinal organoids to a single cell suspension for single cell RNA-sequencing and/or organoid passaging.

59. Remove the organoid medium and replace with 200 μ L of Cell Recovery Solution.
60. Using a p1000 pipette, scrape the gel off the bottom of the plate and pipette up with the cell recovery solution. Transfer to a 1.5 mL Eppendorf tube and incubate on ice.
61. Every 5–10 min, invert the tube and place back on ice to prevent the gel settling at the bottom of the tube.
62. After 20mins on ice, centrifuge the sample at 400 g for 5 min. If Matrigel has not fully dissolved, maintain on ice for further five minutes and repeat until this is the case.
63. Remove supernatant and re-suspend in 500 μ L of pre-warmed TrypLE; incubate for 10 min in a water bath at 37°C. The sample should be assessed under a microscope to assess whether further incubation time is required.

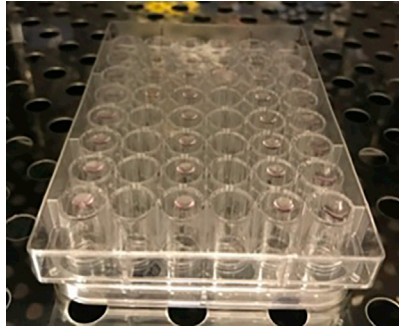


Figure 7. 48 well plate inverted to allow the gel to set and to prevent settling of the cell suspension to the bottom of the well

64. Add 500 μ L of refrigerated DMEM and centrifuge at 400 g for 5 min, remove supernatant and resuspend in 500 μ L of DMEM (4°C). Repeat this step for a total of three times.

Δ **CRITICAL:** It is vital to remove as much of the supernatant as possible to ensure thorough removal of any enzyme mix.

Δ **CRITICAL:** Prior to loading the cells for single cell analysis, it is critical to filter the cells through a 40 μ m filter as described above.

EXPECTED OUTCOMES

Yield of viable cells:

Each digestion will yield a different number of cells, largely depending on the quantity of starting material. In general, one should expect a yield of 5×10^6 – 5×10^7 cells in total, but this number can vary greatly.

Cellular viability should be expected to be in excess of 90%.

Human intestinal organoids as demonstrated in [Figures 8 and 9](#)

LIMITATIONS

This protocol has not been assessed in second and third trimester human fetal gut, however the protocol has been applied to first trimester fetal, neonatal, pediatric, and adult intestinal samples.

Fetal intestinal tissue may be more variable in viability depending on the total ischemic time of the tissue. It is therefore important to proceed with dissociation as soon as is practicable.

TROUBLESHOOTING

Problem 1

Incomplete digestion of tissue after enzymatic treatment.

Potential solution

Incomplete digestion is usually due to a problem with low enzymatic activity. Ensure that the plate is pre-warmed to 37°C prior to commencing digestion and ensure appropriate storage of the enzyme stock and limit repeated thawing of stock solution.

Problem 2

Loss of tissue during processing

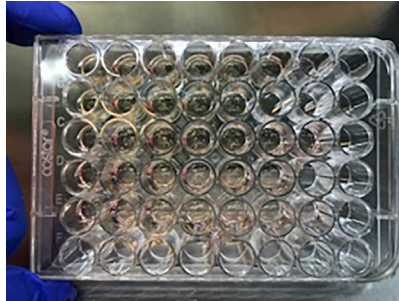


Figure 8. Human intestinal organoids in Matrigel with media applied in a 48-well plate

Potential solution

The volume of tissue is usually low, and thus it is important to preserve it as much as possible during processing. Sometimes the tissue or cells may be “lost”, and this is likely due to sticking of cells/tissue to plasticware. To avoid this, ensure all plasticware is pre-coated with a solution of 0.1% BSA. Particularly important are the mechanical disruption steps, with the pipette; without appropriate coating the tissue can be caught entirely within the pipette tip and therefore be lost entirely if care is not taken.

Problem 3

Low cellular viability of single cell suspension

Potential solution

The protocols described should yield high cellular viability (>90%). However, low viability may occur as a result of either the delayed processing the sample (hours), or as a result of residual enzyme in the processed sample as a result of incomplete washing of the sample post-digestion. It is therefore vital to ensure rapid processing and stringent wash steps after enzymatic digestion.

Problem 4

Clogging of the sample during single cell processing

Potential solution

When a sample is run on a chip in the 10X controller, the sample may clog the system resulting in failed processing. To prevent this occurring, the sample should be filtered immediately before loading, in addition to previous filtration steps.

Problem 5

Loss of Matrigel domes after seeding organoids

Potential solution

If the Matrigel gel is lost after media has been applied, this is unlikely to be due to the gel not having set, but is probably caused by residual enzyme that was not removed due to insufficient wash steps after digestion. It is paramount therefore, to ensure these steps are stringently adhered to, and additional wash steps can be used if necessary.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the technical contact, Professor Matthias Zilbauer (mz304@cam.ac.uk), and by the lead contact, Dr. Alexander D.B. Ross (ar762@cam.ac.uk).

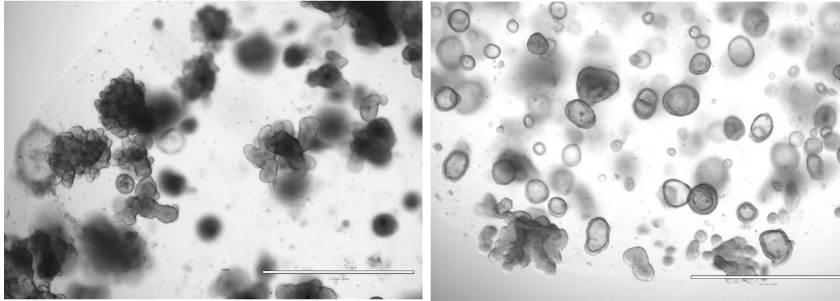


Figure 9. Illustrative images of pediatric intestinal organoids at passage 7 (terminal ileum, left) and fetal intestinal organoids (ileum, right) visualized using a bright-field microscope

Scale bar, 1000 μ M.

Materials availability

Organoid lines generated are available to external researchers following completion of a Materials Transfer Agreement. Please contact Professor Zilbauer for more information.

Data and code availability

The accession number for the raw sequencing data reported in [Elmentaite et al, 2020](#) is E-MTAB-8901.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

A.D.R. developed the protocols and wrote the manuscript. F.P., R.E., and S.A.T. edited the manuscript. M.Z. developed protocols and edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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