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## Isolating human intestinal crypts from biopsies for organoid generation

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**Protocol status:** In development

**We are still developing and optimizing this protocol**

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## Abstract

This protocol provides details on crypt isolation from human terminal ileum and colon to robustly generate organoids.

## Guidelines

Human intestinal biopsies are obtained from endoscopic procedures. All the biopsies are performed after patients' consents and approval from Institutional Review Board at the University of Chicago (IRB Number: 15573A). Intestinal organoids are maintained in human organoid media (details in Materials) and are not prone to differentiation until cultured in differentiation media (unlisted in this protocol).



## Materials

### MATERIALS

- ☒ 100 mg Ciprofloxacin (Cipro) **biorbyt Catalog #orb134677**
- ☒ SB202190 25 mg **Stemcell Technologies Catalog #72634**
- ☒ Nicotinamide **Sigma Aldrich Catalog #N0636**
- ☒ 6-well plate **Corning**
- ☒ 0.5M EDTA solution **Thermo Fisher Scientific Catalog #15575020**
- ☒ Y-27632 **Stemcell Technologies Catalog #72303**
- ☒ DPBS no calcium no magnesium **Thermo Fisher Scientific Catalog #14190144**
- ☒ Advanced DMEM **Thermo Fisher Catalog #12491015**
- ☒ Penicillin-Streptomycin (10,000 U/mL) **Thermo Fisher Catalog #15140148**
- ☒ Glutamax 100x **Thermo Fisher Scientific Catalog #35050061**
- ☒ HEPES 1M **Thermo Fisher Scientific Catalog #15630080**
- ☒ N2 supplement 100x **Thermofisher Catalog #17502048**
- ☒ N-Acetyl-L-( )-cysteine **Fisher Scientific Catalog #O1049-25**
- ☒ EGF recombinant mouse protein **Thermofisher Catalog #PMG8043**
- ☒ Jagge-1 **Anaspec Catalog #AS-61298**
- ☒ A 83-01 **Stemcell Technologies Catalog #72024**
- ☒ CHIR99021 **Stemgent Catalog #04-0004-10**
- ☒ LY2157299 **Cayman Chemical Company Catalog #15312**
- ☒ [Leu15]-Gastrin I human **Sigma Aldrich Catalog #G9145**
- ☒ Recombinant Human R-Spondin-1 **peprotech Catalog #120-38**
- ☒ L-WRN **ATCC Catalog #CRL-3276**
- ☒ Recombinant murine WNT-3A **peprotech Catalog #3115-20**
- ☒ Recombinant human Noggin **peprotech Catalog #120-10C**
- ☒ B27 supplement minus Vitamin A **Thermofisher Catalog #12587010**
- ☒ Matrigel Growth factor reduced **BD Biosciences Catalog #356230**
- ☒ ART™ Wide Bore Filtered Pipette Tips **Thermofisher Catalog #2079G**
- ☒ Cell strainer 100 micron **Corning Catalog #431752**
- ☒ 12-well TC treated plate **Corning**
- ☒ 24-well plate **Corning**



 15 ml corning tube **Corning Catalog #352095**

Dissociation medium 100 ml

8 mM EDTA in DPBS without Calcium and Magnesium

Complete ADF media with conditioned media is composed of:

- a. Advanced DMEM/F12
- b. 1X Glutamax
- c. 10mM HEPES
- d. 1X Pen/Strep
- e. 1X N2 supplement
- f. 1X B-27 Supplement Minus Vitamin A
- g. N-acetyl-L-(+)-cysteine (1.25 mM)
- h. Nicotinamide (10 mM final)
- i. 50% L-WRN conditioned media
- j. Murine EGF (50 ng/ml)
- k. Jagged-1 (1 uM)
- l. Y-27632 (10 uM)
- m. SB202190 (30 uM)
- n. A-8301 (500 nM)
- o. Chir99021 (2.5 uM)
- p. LY2157299 (500 nM)
- q. Leu15-Gastrin I (10 nM)
- r. Recombinant human R-spondin1 (500 ng/ml)

Alternatively, only with recombinant proteins, complete ADF media is composed of:

- s. Advanced DMEM/F12
- t. 1X Glutamax
- u. 10 mM HEPES
- v. 1X Pen/Strep
- w. 1X N2 supplement
- x. 1X B-27 Supplement Minus Vitamin A
- y. N-acetyl-L-(+)-cysteine (1.25 mM)
- z. Nicotinamide (10 mM)
- aa. Murine Wnt3A (100 ng/ml)
- bb. Murine epidermal growth factor (50 ng/ml)
- cc. Noggin (100 ng/ml)
- dd. R-spondin-1 (500 ng/ml)
- ee. Jagged-1 (1 uM)
- ff. Y-27632 (10 uM)
- gg. SB202190 (30 uM)
- hh. A-8301 (500 nM)
- ii. Chir99021 (2.5 uM)



jj. LY2157299 (500 nM)

kk. Leu15-Gastrin I (10 nM)

Ciprofloxacin 10 ug/ml is added freshly when feeding cells.

Stocks of small molecules and recombinant proteins are prepared and stored according to the manufacturer's instruction.

P1000 tips

Customize the pipet tip. If needed, tips can be altered by cutting it to slightly smaller than the size of the largest biopsy, using a sterilized razor blade. Cut the pipet tip at a straight angle, not biased, to keep the hole as small as possible.

## Before start

Human organoid media also listed as complete Advanced DMEM/F12 (complete ADF) is supplemented with L-WRN (murine cell line) conditional media, alternatively complete ADF can be supplemented with recombinant proteins.



- 1 Collect biopsies in PBS or culture media in 1.5 ml Eppendorf tubes.
- 2 Prechill PBS and 8mM EDTA in PBS on ice. Pre-warm human organoid medium and 12-well/24-well plate in the tissue-culture incubator.
- 3 Once biopsies received, wash 3 times with 25 ml ice cold PBS each in 50 ml conical tubes, or until PBS looks clear.

#### Note

Biopsied tissues range from 6 to 20 mm<sup>3</sup> in size. All the tissues biopsied from the same site are pooled for downstream procedures.

- 4 Incubate all the tissues from each biopsy in 25mL of 8 mM EDTA/PBS in a 50 ml conical tube for 30 min, rocking (not shaking) at 4C.

🌡 4 °C

🕒 00:30:00

- 5 Place one 100 um cell strainer on each well of a 6-well plate. Pre-wet the cell strainer with 1mL culture media.
- 6 Transfer biopsies from EDTA/PBS into 1-2 ml ADF.
- 7 Using a customized P1000 *aerosol resistant* tip, pipet up and down vigorously, making sure that the biopsy tissue goes in and out of the tip, but with some difficulty. This helps release the crypts.

#### Note

Customize the pipet tip: tips can be altered by cutting it to slightly smaller than the size of the largest biopsy, using a sterilized razor blade. Cut the pipet tip at a straight angle, not biased, to keep the hole as small as possible.

- 8 Use the 100 um cell strainer to filter the tissue and large pieces from the crypts. Collect the crypts into a 1.5 ml microfuge tube. Check to see whether enough crypts were released from



the tissue on a microscope.

- 9 Centrifuge the crypts at 300-400 g at 4°C for 5 min in a tabletop swinging bucket centrifuge



300 x g, 00:05:00



4 °C

#### Note

A microcentrifuge is not recommended here because it is a fixed angle and we want the pellet to be at the bottom of the tube, not on the side

- 10 Remove the supernatant and resuspend the crypts in pre-warmed and CO<sub>2</sub>-equilibrated human organoid media (complete ADF) containing growth factors

- 11 Mix crypts with thawed matrigel in a ratio of 1:2 (cells:matrigel). Pipet up and down; avoid creating bubbles

- 12 Plate crypt/matrigel mixture onto a pre-warmed 12- or 24-well tissue culture plate, 100 or 50 µl/well respectively.

- 13 Incubate 45-60 min in 37°C, 5% CO<sub>2</sub> incubator



37 °C



00:45:00 Incubation

- 14 Add complete ADF into each well (24-well: 0.5 ml/well; 12-well: 1 ml/well)

- 15 Feed organoids with complete ADF every other day. Also add ciprofloxacin (10 µg/ml) to make sure that there are no remaining bacteria in your culture.

#### Note

Media is changed every other day. Organoids are split and expanded as needed, about once per week.