ORGANOID ISOLATION – large intestine

Crypt Isolation Media
DMEM/F12 Advanced
Pen/Strep
Glutamine or Glutamax
HEPES (10mM final)

Crypt Growth Media (ENR50)
DMEM/F12 Advanced containing Pen/Strep, Glutamine, HEPES
40 ng/ml Epidermal Growth Factor (Murine Recombinant, Peprotech 315-09),
50 ng/ml Noggin (Murine Recombinant, Peprotech 250-38)
250ng/ml R-spondin 1 (R&D Systems 3473-RS) or ***conditioned media***
100ng/ml Wnt3a (Millipore GF-160) or ***conditioned media***

For 100ml of media:
20 ul of EGF
100 ul of Noggin
5ml conditioned media
25ml conditioned media

NOTE: This recipe uses ~50% the amount of R-spondin and Noggin originally published in Sato et al, 2009. I have maintained wild-type organoids in this media for 6 months without noticeable changes in cell behavior.

On Ice:
Sterile PBS
Sterile Filtered 5mM EDTA/PBS
Matrigel (BD 354234)

1) Euthanize mouse, spray (drench) with 70% EtOH and move into the TC hood. Remove entire colon while avoiding the skin and hair, and trim away as much mesentery as possible.
2) Open longitudinally and scrape away excess mucous and fecal matter using a glass slide. Place in 20ml of PBS on ice. Shake vigorously to remove any fecal material. Pour off PBS and repeat until the supernatant is clear – approx. 4 washes
3) Cut into ~5-10mm pieces and place in 10ml of ice-cold 5mM EDTA/PBS. Pipette vigorously up and down using 10ml pipette (~15 times). Remove the supernatant.
4) Add 10ml of EDTA/PBS, Invert 15 times then agitate gently (rocker, roller, shaker) for 15 minutes at 4C.Remove supernatant and wash once with ice-cold PBS.
5) Add 3ml of basal medium containing 500U/ml Collagenase IV. Pipette up and down 5 times using a 5ml pipette and place in a 37C water bath for 30mins
6) Crypt Releasing Step:
    a. Add 10ml PBS and pipette vigorously ~15 times. Collect supernatant containing villi fragments and crypts as Fraction 1.
    b. Repeat 5a and collect the supernatant as Fraction 2.

NOTE: In our hands Fraction 2 always contains the best ratio of crypts : villi/debris, but this will depend on multiple factors and should be determined individually for each lab / user. It may be necessary to collect a third or even fourth fraction, depending on initial results.

7) Add 10ml Crypt Isolation Medium containing DNAse I (200U), pipette up and down gently to mix and pour (do not pipette) through a 100μm filter. You may have to use 2-3 filters as they may clog depending on amount of debris.
8) Filter flow-through on a 70um filter into a 50ml conical tube containing 1ml of FBS.
9) Spin at 1000rpm for 4 minutes.

10) Resuspend in ~150µl Crypt Isolation Media and check under the microscope.
Crypts appear as thin U-shaped structures. If you have access to Lgr5-EGFP mice, it can be useful to first run the isolation using these animals as crypts are readily identified by GFP staining at the base.

11) Add Matrigel to a small volume of crypts – if possible try to keep the volume you add as small as possible (5ul crypts/100ul of Matrigel works well, but you can double that if crypts are sparse). Keep Matrigel on ice at all times.

12) Pipette Matrigel droplets into multiple small wells. We commonly use 40ul in each well of a 48 well plate and establish 4-6 wells per isolation – occasionally you will develop fungal or bacterial contamination. Spreading the sample over multiple wells ensures low-level contamination does not affect the entire sample. Let polymerize for 5-10 minutes in the hood, then harden in a 37^{oC} incubator for 15 minutes.

13) Add 250 ul of Crypt Growth Media over the gel. Replenish WENR50 media every 2 days.