ORGANOID ISOLATION – large intestine

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

Post-isolation media (ENYCF)
Crypt isolation media +
40 ng/ml Epidermal Growth Factor (Murine Recombinant, Peprotech 315-09),
50nM LDN-193189 (Selleckchem, Catalog No.S2618)
10uM Y27632: ROCK inhibitor (Sellekchem, Catalog No.S1049)
5uM CHIR99021 inhibitor (CAYMAN CHEMICALS - 13122)
10ug/ml FunginTM (InvivoGen, #ant-fn-1)

Crypt Growth Media (WENR)
Crypt isolation media +
40 ng/ml Epidermal Growth Factor (Murine Recombinant, Peprotech 315-09),
50nM LDN-193189 (Selleckchem, Catalog No.S2618)
1:20 RSPO1 conditioned media
0.5nM Fc-WNT (ImmunoPrecise, N001 - 100µg/500 µg)

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 colon into 5-10mm pieces into ice cold EDTA/PBS (10ml) in 50 ml Falcon tube. Place on shaker / roller at 4°C for 60 minutes. In this time prepare falcon tube with filters
4) Shake tube vigorously ~10 times and remove supernatant. Replace with ice cold sterile PBS + 200 U DNaseI (20ul/10ml for one fraction) and shake vigorously ~30 times (fraction I). Repeat once (fraction II).
5) Filter fractions twice through a 100µm filter into a 50ml conical tube containing 1ml of FBS
6) Spin at 1000rpm for 4 minutes.
7) 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.
8) 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.
9) 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.
10) Add 1ml of EN+Y+Chir+Fungin for 2 days, check the following day for contamination and then after two days swap to WENR media. Replenish WENR media every 2 days.