**Isolation of crypts from small intestine**

On Bench

1. Remove entire small intestine (from end of stomach to beginning of colon) and place on paper towel that has been moistened with 1x PBS
2. Clean the remaining fat off and cut the intestine into 3 pieces
3. Working on one piece at a time, cut open longitudinally using scissors, and opening it up with forceps
4. Maintaining the interior surface up, place the opened intestine fragment onto a 150mm plate with few drops of 1x PBS
5. Gently scrape off the fecal matter and villi w/blade
6. Place the piece in 25mL of cold 1x PBS
7. Proceed with the other two intestinal pieces as with the first
8. Take pieces out of conical tube with forceps and place them in fresh 25mL of 1x PBS+ 250ul of 0.5M EDTA [5mM]F on ice for 5’
9. After the 5’ shake conical tube vigorously
10. Remove the pieces and place in another 50 ml conical tube containing fresh 25mL of 1x PBS+ 250ul of 0.5M EDTA [5mM]F
11. Place conical on twirly rocker in the 4C cold-room for 30’-45’
12. Vigorously shake tubes and remove remaining intestine pieces with forceps (these are trash). Add up to 50mL of cold 1x PBS to dilute out the EDTA
13. Spin down supernatant 5’ @1300.
14. Wash the pellet again with 1x PBS and spin again. The pellet now contains villi, lymphocytes, other single cells and crypts

In Hood

1. Aspirate the supernantant and resuspend the pellet in 10 mL of cold 1x PBS
2. Filter the cell suspension through 70uM mesh
3. Spin down 5’ @500
4. Resuspend the pellet in 2mL 1x PBS

The crypts are plated on pre-warmed 48-well plate. 100-140ul of crypts derived from one animal with 30% Matrigel (BD 356231) for a total of 200ul per well.

Let the Matrigel/crypt mixture solidify at 37C incubator for 20-30’ before adding AdMEM+ medium.

Crypt/Organoid medium (AdMEM+):

Advanced DMEM/F12 (Invitrogen 35050-068)

1x Antibiotic-Antimycotic [100x]i (Invitrogen 15240062/Gibco 15240)

1x N2 supplement [100x]i (Invitrogen 17502-048)

1x B27 supplement [100]i (Invitrogen 17504-044)

N-Acetylcysteine [500mM]i (Sigma-Aldrich A9165-5G)

50ng/ml EGF [100ug/ml]i (PeproTech 315-09)

100ng/ml Noggin [100ug/ml]i (PeproTech 250-38)

500ng/ml Rspondin [100ug/ml]i (R&D Systems 4645-RS)