Small and large intestine prep (Gut Prep)

-Resect the entire small/ large intestine in one piece. Place in 50 mL conical tube containing PBS or 1X DMEM **w/o phenol red**. Media should have glucose in it so DMEM or RPMI with no phenol red will work.

-Clean the fat or remaining mesenteric lining from the intestine, removal of the fat is crucial to get a good yield. Be very thorough!

-Remove **all peyer's patches** from the small intestine (~8-12 in each small intestine, can vary with different strains). These look like small white masses that are located on the opposite side of the mesentery.

-After all peyer's patches have been removed, cut the intestine length-wise and clean intestinal contents by gently moving the intestine back and forth in a Petri dish full of media or 1XPBS.

-Cut the intestine into 1-3 cm pieces and place the pieces in a 50 mL falcon tube containing 25 mL of media plus EDTA and DTT (final concentration of DTT and EDTA is 0.145 mg/mL and 5 mM, respectively.)

-Secure the lid by wrapping with parafilm and place tube in a 37°C bacterial shaker (200-207 rpm) for 40 min. While this is shaking you should make the media containing the enzymes.

-Add 5 mg of **DNAse**, 50 mg of **Collagenase D** or **Collagenase IV** and 10 mg of **Liberase TL** (2 vials of the Liberase TL) in 50 mL of 1x DMEM (no phenol red). You will use 25 mL of this media per intestine. Remove 0.5 mL of media from each 25 mL reaction and aliquot into 1.5 mL eppendorf tubes. At this point you should have 1 eppendorf tube with 0.5 mL of media and 1 (50 mL) falcon tube with 24.5 mL of media per intestine.

-**To isolate lamina propria lymphocytes proceed to the next step.** If you are interested in collecting intra-epithelial lymphocytes (IELs) you should 1st pour the contents of the tube into a 50 mL conical tube, using a 70 or 100 micron falcon cell strainer. Centrifuge the filtered suspension at 4°C for 10 min at 1500 rpm. Re-suspend the pellet in 25 mL of complete media and strain through a 40 micron cell strainer. Centrifuge filtered cells as above. You could purify the mononuclear/lymphocyte population by using ficoll-hypaque gradient centrifugation. Otherwise, the cells are ready for analysis at this point.

-Drain the tube contents into an aluminum mesh sieve placed on top of a glass beaker. In this step we are interested in collecting the pink lamina propria tissue fragments that remain in the sieve. Add these pieces back to a 50 ml tube with 10 ml of media only and shake the tube by hand for ~30 sec then repeat the strainer step. Remove these pink tissue fragments from the mucus/epithelial

layer and place in a 1.5 mL eppendorf tube containing 0.5 mL of the media plus enzymes. After removing all of the pink lamina propria tissue tissue, mince the tissue in the eppendorf tube using fine scissors.
-Dump the minced pieces into the falcon tube containing the 24.5 mL of media and seal lid with parafilm. Place the tubes in the bacterial shaker and agitate for 15-20 minutes. The new Liberase TL enzyme is very effective, thus the digestion occurs within this period of time, given that you have carefully minced the tissue. **Important note: clean the sieve after every sample to prevent intra-sample contamination.**

-Place a 100 micron falcon cell strainer on top of a 50 mL falcon tube. Strain the digested tissue. Fill the tube with RPMI containing 10% FCS and spin at 4°C for 10 min at 1500 rpm. Resuspend in 10 ml of media then pass through a 40 micron filter and spin again. The cell pellet will contain lamina propria lymphocytes along with epithelial and other cells. You are now ready to do analysis on these cells. For cytokine expression analysis, you need to culture these cells in a 96 round bottom plate with PMA/ionomycin (50 ng/mL, 1 μM) + Golgi plug (BD Fix/Perm kit, use at 1 μL/mL) for 4-5 hrs.

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