

Isolation of Human Crypts - Long Method

(Modified it from mouse Sato method)

1. Cut mucosa into 1 x 1 cm pieces.
2. Wash mucosa with 30 ml PBS in 50 ml tube, vortex for 30 seconds (3 pulses of 10 second duration) and remove supernatant repeat about 8-15 times.
3. Add 30 ml PBS containing 2.5 mM EDTA and 1mM DTT and then Rock sample at 4 °C for 30 minutes.
4. Make every effort to remove all supernatant.
5. Add PBS to a total volume of ~25–30 mL and vortex gently for 30 Sec. in 3 sec pulses. Transfer supernatant to a 15 mL tube after tissue settles down. Repeat 6 times until most of crypts are released.
6. Spin down the crypt fractions at 140 rcf and 4 °C for 2 min and remove supernatant (leave 2 mL in tube to re-suspend).
7. Select tubes 2-6 (If all tubes have significantly large pellets, then select tubes 3-6) to re-suspend. Add FBS to each tube to make the solution 10%FBS by volume and mix up and down by transfer pipette.
8. Combine fractions (tubes) 2-6 or 3-6 and filter through 100 um and 70um cells strainer.
9. Centrifuge filtrate at 140 rcf and 4 °C for 2 min to form pellet.
10. Remove all supernatant and re-suspended pellet in 5mL of Basic Media.

Isolation of Human Crypts - Short Method

1. Wash whole piece of mucosa (2~4g) with 30 ml PBS in 50 ml tube, vortex for 1 minute and remove supernatant by lifting mucosa with forceps and pour to the waste container repeat about 8 times.
2. Add 30 ml PBS containing 2.5 mM EDTA and 1mM DTT and then Rock sample at 4 °C for 30 minutes.
3. lift mucosa with forceps and pour out all supernatant to the waste container with bleach.
4. Add 15ml of PBS to vortex it for 30~60 Sec. and lift mucosa with forceps and pour all supernatant into a 15 ml tube (first fraction).
5. Let first fraction settle down on ice for about 5 ~ 10 min. Then discard supernatant to remove single red blood cells.
6. Re-suspend the fraction in 10ml PBS by inverting 5 times. Let it settle down on ice for 30~60 Sec. Then filter the crypts in suspension through a 70um strainer, leaving the pellet.
7. Repeat step 6 if need more crypts.
8. Centrifuge filtrate at 140xg and 4 °C for 2 min.
9. Remove all supernatant and re-suspended pellet in 5ml of Basic.
10. Count crypts and plate them with Matrigel to 48-well plate, 200 crypts in 25ul Matrigel per well.

Comparison of different between these two methods

Short Method

- Super pure crypts -- Separates and removes single cells and villi from the crypts. Uses fewer fractions, preventing accumulation of cellular debris.
- High yield -- Improved filtration step after removal of single cells and villi from prep.
- High efficiency of cell growing
- Process -- 1.5 ~ 2 hours (Shorter washing steps – whole mucosa is lifted and supernatant is decanted. Only one centrifugation step. Cleaner procedure more compatible with working in biosafety cabinet.

Long Method

- Impure crypt prep -- filtration step does not remove all debris or single cells from the prep. Excess cellular debris collected in fractions 2-6 or 3-6.
- Low crypt yield -- Majority of crypts in fractions 1 and 2 were discarded. Clogging of filter with villi led to crypt loss.
- Low efficiency of cell growing -- impure and broken crypts were decreased number of cells grow.
- Process: 3 ~ 4 hours -- Cutting mucosa into 1 x 1 cm pieces created difficulty for removing supernatant during washing, requiring more washing steps and time)

Conclusion: Short method is easier, faster, and higher yield than the long method.

Short Method to isolate Human Crypts (8/28/15)

