

Murine Crypt Isolation & Organoid Passaging
Breault Lab HDDC Organoid Core Protocols
Updated: 2/12/2018

Reagents	Equipment
Fresh 1X Sterile PBS	Clean cell cultures dishes
Fresh EDTA in PBS	15mL and 50mL conical tubes
Advanced DMEM/F12 (Gibco)	70um cell strainer
Matrigel matrix (Corning)	
Pre-warmed culturing medium	

Crypt Isolation

DUODENUM

- Keep 1X PBS and **2mM** EDTA/PBS on ice during procedure
- Euthanize adult mouse with CO₂ followed by cervical dislocation
- Dissect open mouse, locate start of small intestine, cut ~1cm after stomach, remove ~10cm of proximal duodenum. Move to dish of cold 1X PBS.
- Using a sterile syringe, tubing, and tip, flush intestine with cold 1X PBS until cleared of stool and debris
- Remove any extraneous connective tissue on outside of intestinal section
- Using small scissors (blunt tip work best), filet intestinal section to expose epithelium
- Wash intestinal section in dish of PBS to remove residual stool and debris
- With a sterile new razor blade, dice into ~5mm pieces
- Place diced intestine into 50mL conical tube with ~25mL cold 1X PBS
- Swirl to wash, allow pieces to settle, and decant; repeat at least 10X until PBS is clear
- Decant PBS, pipet off residual. Add 25mL fresh 2mM EDTA
- Place tube horizontally on ice, and rotate for **15 minutes**.
- Allow pieces to settle, decant EDTA, and replace with 25mL fresh 2mM EDTA.
- Place tube horizontally on ice and rotate for **25 minutes**.
- Remove tube from ice, shake vigorously for **1 minute** to release crypts from tissue. Solution will become very cloudy.
- With a 10mL serological pipet, pipet up and down 25x while mixing.
- Pass solution through 70um filter into new 50mL conical tube.
- Spin at 300 x g for **5 minutes**.

COLON

- Keep 1X PBS and **5mM** EDTA/PBS on ice during procedure
- Euthanize adult mouse with CO₂ followed by cervical dislocation
- Dissect open mouse, locate cecum, and remove entire colon from cecum to rectum. Move to dish of cold 1X PBS.
- Using a sterile syringe, tubing, and tip, flush intestine with cold 1X PBS until cleared of stool and debris
- Remove any extraneous connective tissue on outside of intestinal section
- Using small scissors (blunt tip work best), filet colonic section to expose epithelium
- Wash intestinal section in dish of PBS to remove residual stool and debris

- With a sterile new razor blade, dice into ~5mm pieces
- Place diced intestine into 50mL conical tube with ~25mL cold 1X PBS
- Swirl to wash, allow pieces to settle, and decant; repeat at least 10X until PBS is clear
- Decant PBS, pipet off residual. Add 25mL fresh 5mM EDTA
- Place tube horizontally on ice, and rotate for **15 minutes**.
- Allow pieces to settle, decant EDTA, and replace with 25mL fresh 2mM EDTA.
- Place tube horizontally on ice and rotate for **30 minutes**.
- Remove tube from ice, shake vigorously for **1 minute** to release crypts from tissue. Solution will become very cloudy.
- With a 10mL serological pipet, pipet up and down 25x while mixing.
- Pass solution through 70um filter into new 50mL conical tube.
- Spin at 300 x g for **5 minutes**.

STOMACH

- Keep 1X PBS and **10mM** EDTA/PBS on ice during procedure.
- Euthanize mouse with CO₂ followed by cervical dislocation.
- Dissect open mouse, locate, and remove stomach. Move to dish of cold 1X PBS.
- Dissect stomach, open along greater curvature, and wash in 1X PBS.
- Remove serosal muscular layer, pinning if necessary.
- With a sterile new razor blade, dice desired sections into ~5mm pieces
- Place diced gastric gland into 50mL conical tube with ~25mL cold 1X PBS
- Swirl to wash, allow pieces to settle, and decant; repeat at least 10X until PBS is clear
- Decant PBS, pipet off residual. Add 25mL fresh 10mM EDTA
- Place tube horizontally on ice and rotate for **2 hours**. Replace with fresh 10mM EDTA halfway.
- Remove EDTA and add dissociation buffer (43.4mM sucrose and 54.9mM D-sorbitol in PBS).
- Shake vigorously for **2 minutes**.
- With 10mL serological pipet, pipet up and down 25x while mixing.
- Pass solution through 70um filter into a new 50mL conical tube.
- Spin down at 300g for **5 minutes**.

Crypt Plating

The following steps should be performed in a biosafety hood

- Aspirate EDTA, resuspend pellet in 20mL Advanced DMEM/F12.
- Spin at 300 x g for **5 minutes**. Repeat 2x for a total of 3 washes.
- Add appropriate volume of Matrigel matrix to 15mL conical tube.
50uL of Matrigel per well in a 24 well plate
- Add 1uL crypt pellet per 50uL Matrigel expecting to plate.
Plating concentration typically 50-100 crypts per well, 1uL of crypt pellet is ~50-100 crypts. For 12 wells, add 12-15uL of crypt pellet to 625uL Matrigel
- Suspend crypts into Matrigel with P1000, avoiding bubbles
- Plate 50uL of Matrigel suspension into each well. Incubate at 37°C for **10 minutes**.
Allows Matrigel to polymerize. For dense cultures, 15-20 minutes is appropriate.
- Add 500uL pre-warmed media to each well. Incubate organoids at 37°C/5% CO₂.
Feed after 4 days.

- Feed and passage as needed.
Feed every 2-3 days, passage every 7-10 days.

Organoid Passaging

Typically confluent cultures will split 1:3 or 1:4

- Aspirate media from wells.
- Add 500uL Cell Recovery Solution (Corning) to each well.
- Scrape Matrigel domes off plate into solution with P1000, move to 15mL conical tube, combining wells as necessary.
- Incubate on ice for **40 minutes**.
- Invert tube gently a couple times, spin at 400 x g for **5 minutes**.
- Aspirate supernatant, add appropriate amount of Matrigel.
- Using a bent P1000 tip, pipet up and down ~50x to break up organoids and resuspend pellet into Matrigel.
- Plate 50uL Matrigel suspension into each well. Incubate at 37°C for **10 minutes**.
- Add 500uL pre-warmed media to each well. Incubate organoids at 37°C/5% CO₂.
Feed after 4 days.
- Feed and passage as needed.