**Single cell prep from mouse stomach for FACS sorting**

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**Reagents:**

1. EDTA 0.5M, pH 8.0 – Invitrogen AM9260G

2. Accumax – Innovative cell technologies AM105

3. Dispase – Stem Cell Technologies – 07913

4. TrypLE 10x – Thermo Fisher A1217701

5. Y-27632 (ROCK inhibitor)- 10mM stock, 1:1000 - Stem Cell Technologies 72304

6. DNase I recombinant – Roche 04 716 728 001 - 1:1000

**Protocol:**

1. Dissect abdominal organs of mouse and isolate the entire stomach. Cut while maintaining 1-2cm of adjacent esophagus & duodenum at either end.

2. Pin the stomach on a dissection dish and cover with cold PBS. I generally use 1-2 pins on the forestomach and 1 pin on the duodenum (since I don’t use these part) to hold the stomach flat and slightly taut against the dissection dish.

3. Remove attached soft tissue and vasculature from the stomach wall using your finger or blunt forceps.

4. Identify the gastro-intestinal boundary. A slightly compressed pyloric sphincter is easily visible to help appreciate the boundary. In addition, the serosa for distal stomach and proximal intestine have slightly different hues which makes the boundary easy to identify. Then trim the small intestine to remove from stomach. Trim a 1-2mm cuff of the distal stomach with the small intestine to remove any chances of contamination by intestinal cells.

5. Butterfly open the stomach along the greater curvature and wash with PBS and pin down again so as to have the mucosa facing up. You can macroscopically discern Corpus from Antrum (image below from Mahe et al. [Curr Protoc Mouse Biol. 2013 Dec 19; 3: 217–240.](https://www.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&retmode=ref&cmd=prlinks&id=25105065)) Cut out antrum and try and keep it as 1 piece of tissue. Alternatively, if the entire hind stomach or corpus is required dissect appropriately.

An external file that holds a picture, illustration, etc.
Object name is nihms557832f4.jpg

6. Wash tissue in PBS and then transfer to 20ml of 10mM EDTA (in PBS) at room temperature. Rotate at room temperature for 20 minutes. Some glands will be released at this point but most of them are still attached to the stomach. EDTA treatment will allow easy removal of glands in the next step.

\* If processing whole hind stomach or corpus add DTT (final conc 5-10mM) to the EDTA solution to act as a mucolytic.

7. Place tissue (mucosa facing up) on a 3cm petridish and cover with 1-2cc of PBS. Using two glass slides gently scrape the gastric mucosa. Small chunks of tissue will be released into the PBS. Gently pipette using P1000 to homogenize and transfer to a conical. Generally, 1-3 scrapes are sufficient to release all the glands.

\*You should check the petridish under the microscope to ensure whole gastric glands are seen. Typically, you will be abundant glands in solution after scraping.

8. Pellet the gastric glands at 200g x 5 mins at 4 degrees. Discard the supernatant and resuspend in a 50cc conical in 20-30ml of ADT (Accumax, Dispase and 10x typLE mix – 2.5cc of Accumax, 2.5cc of dispase, 0.5cc of TrypLE with 4.5cc of phenol red free DMEM in 10ml of ADT solution) to digest to single cells.

\* Add ROCK inhibitor (10uM final concentration) and DNase I (1:1000) to the enzyme mix. Ggiven the short digestion time addition of DNase helps disaggregate cell clumps.

\*Make sure the enzyme mix is pre-warmed to 37 prior to suspending glands in it. Incubate at 37 degrees (warm room) for 8-10 minutes on rotating shaker. Using a 5ml pipet 2-3 times every 3-4 minutes to allow for reasonable dissociation to single cells within this time frame. Longer digestions invariably affect yield. **It is critical to visualize efficacy of dissociation under the microscope along the process**. The goal is to obtain ~30-50% cells as single cells and remaining as very small clumps which can be broken manually at the time of resuspension.

10. Inactivate enzyme by diluting with DMEM+10% FBS. Spin down filtrate at 1500rpm x 5mins at 4 degrees. Resuspend the pellet in 1-2cc of ice cold FACS buffer (1mM EDTA, 4% FBS, ROCK inhibitor in Phenol red free DMEM).