*Customer Developed Protocol*

# Cell Dissociation and Crypt Isolation of the Mouse Small Intestine

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## Contributor Research Profile

The Regev laboratory studies biological circuits, gene regulation, and evolution. Lab members pursue multi-disciplinary projects that aim to determine how complex molecular circuits function and evolve in response to genetic and environmental changes, cellular differentiation, evolution, and disease. Aviv Regev and her colleagues develop experimental and computational approaches to systematically decipher the mechanisms that underlie the transcriptional regulatory circuits in organisms ranging from yeast to humans.

Link to the lab: https://www.broadinstitute.org/regev-lab

## Reference

This protocol was used in:

Haber *et al.* A single-cell survey of the small intestinal epithelium, *Nature*, 2017, 551(7680):333-39
https://www.ncbi.nlm.nih.gov/pubmed/29144463

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## Required Buffers and Reagents

1. PBS (cold)
2. 20 mM EDTA-PBS
3. TrypLE express (Invitrogen)
4. 40-µm filter
5. 0.4% BSA–PBS
6. FACS machine and cell stain

## Protocol

### Cell dissociation and crypt isolation

The small intestine of C57BL/6J wild-type, Lgr5–GFP or Gfi1b–GFP mice was isolated and rinsed in cold PBS. The tissue was opened longitudinally and sliced into small fragments roughly 2 mm in length. The tissue was incubated in 20 mM EDTA–PBS on ice for 90 min, shaking every 30 min. The tissue was then shaken vigorously and the supernatant was collected as fraction 1 in a new conical tube. The tissue was incubated in fresh EDTA–PBS and a new fraction was collected every 30 min. Fractions were collected until the supernatant consisted almost entirely of crypts. The final fraction (enriched for crypts) was washed twice in PBS, centrifuged at 300g for 3 min, and dissociated with TrypLE express (Invitrogen) for 1 min at 37 °C. The single-cell suspension was then passed through a 40-µm filter and stained for FACS for scRNA-seq (below) or used for organoid culture. We confirmed the robustness of this method by testing additional single-cell isolation methods—either 'whole' (scraping the epithelial lining) or 'villus-enriched' (fraction 1; see above)—and found that, owing to the high mortality rate (via anoikis) of post-mitotic differentiated cells (the primary component of which is mature enterocytes), crypt-enriched single-cell suspension faithfully represents the composition of the types of small intestine cell (data not shown).

### Cell Sorting

A FACS machine (Astrios) was used to sort single cells into an Eppendorf tube containing 50 µl of 0.4% BSA–PBS and stored on ice until proceeding to the GemCode single-cell platform.