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Whole-mount immunostaining of the adult *Drosophila* gastrointestinal tract

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Abstract

The gastrointestinal (GI) tract harbors an essential barrier epithelium that separates an organism from its changing external environment. As such, the gut epithelium is a fascinating nexus of stem cell biology, immunology and physiology. Investigators have sought to mine this rich interface for new biological and mechanistic insights. Many of the powerful genetic approaches developed in *Drosophila* have proven effective in the study of the gut. The goal of this article is to present a method for dissecting, immunostaining and mounting samples of the adult *Drosophila* GI tract. This protocol combines readily with techniques to label cell lineages and/or challenge the system with environmental perturbations, which are briefly discussed.

Keywords

Adult *Drosophila* midgut; Stem cell; Lineage tracing; Environmental challenge

1. Introduction

1.1. Overview of the adult gastrointestinal tract

The adult *Drosophila* gastrointestinal (GI) tract is a tubular alimentary canal consisting of the foregut, midgut and hindgut (Fig. 1). Auxiliary structures include the salivary glands, crop and malpighian tubules, which all enter the GI tract at stereotyped positions along the anterior–posterior axis. Anatomically, the adult midgut is defined by two landmarks: the cardia and the pylorus. In the anterior, the bulbous cardia marks the junction between the foregut and midgut. The cardia functions as a one-way valve and is the site of peritrophic matrix production. In the posterior, the pylorus marks the site where the midgut, hindgut and malpighian tubules all converge in a common duct.

The adult midgut is of endodermal origin but must be clearly distinguished from its antecedent in the embryo [1]. During early development, endodermal rudiments are specified at the embryonic poles, one in the anterior, one in the posterior. These cells invaginate and migrate along visceral muscle tracks through the central axis of the embryo as mesenchyme. Migrating endodermal cells meet in the middle to form the embryonic gut.

As mesenchyme condenses on the visceral muscle substratum to form the gut tube, cells fated to differentiate as embryonic midgut can be distinguished from adult midgut precursors (AMPs). AMPs are evident as individual cells dispersed throughout the gut epithelium, but expand in number throughout larval stages giving rise to cell clusters. During late larval and early pupal stages AMPs coalesce to form the adult midgut, in a process that coordinates shedding of the embryonic midgut into the lumen. Thus, the adult midgut epithelium is utterly distinct from the embryonic midgut and forms *de novo* from AMPs during pupal stages.

At the tissue level, the adult GI tract can be broadly divided into two layers: an outer layer of circumferential and longitudinal visceral muscle, and an inner epithelial monolayer [2]. Two primary differentiated cell types have been identified throughout the midgut, absorptive cells and secretory cells. Their unique morphologies and molecular profiles have now been well defined. The monolayer displays significant regional heterogeneity and is organized into a succession of adjacent segmental territories. Epithelial cells impart distinct physiologies to the gut, such as the striking variation in luminal pH seen in the middle of the midgut.

Differentiated cells lost from the adult GI tract due to injury can be rapidly replenished [3]. Assays for self-renewal and multipotency show that tissue homeostasis is maintained by resident epithelial stem cells. Gut stem cells are distinguished based on position, gene expression, rate of proliferation and cell lineage. Basally, stem cells contact the basement membrane and extend a single apical process toward the gut lumen.

In summary, the adult midgut provides a tractable experimental model to study a wide variety of processes occurring at the interface of an organism and its environment, using molecular genetic approaches. Here, we describe a method for isolating and immunostaining adult GI samples for whole-mount analysis, a key element in performing such experiments.

2. Whole-mount immunostaining of the adult GI tract

2.1. Preparation

In preparation for dissection, adult flies are sorted, their external appendages are removed and they are briefly dehydrated. This process minimizes the amount of tissue in the dissection dish and eliminates any air bubbles, which tend to make the dissection more challenging.

2.1.1. Materials

- Adult *Drosophila melanogaster* (Bloomington Stock Center).
- Yeast paste (freshly hydrated active dry yeast; Red Star).
- Dissecting microscope with zoom and dual goosenecks to supply oblique illumination; CO₂ equipped fly sorting station (Leica MZ16; custom fabrication).
- Forceps, Dumont #5 Dumostar-Biology, 11 cm, 0.05 mm × 0.02 mm (FST #1129510).
- Moria instrument case (FST #20311-21).

- Dumont forceps sharpening stone (FDT #29008-22).
 - Watch glass (Carolina #FA-74-2300).
 - Phosphate buffered saline, 0.01 M (PBS: NaCl, 0.138 M; KCl, 0.0027 M; pH 7.4; Sigma #P3813).
 - Distilled, deionized H₂O.
 - 95% ethanol.
 - Kimwipes.
 - Dissecting dish (14 mm plastic petri dish cast with layer of black sylgard 170 silicone elastomer; Dow Corning).
 - Austerlitz minuten pins; 0.15 mm, black enameled (FST #26002-15).
1. Inspect forceps under dissecting microscope. Ensure that they are straight and that the tines come into apposition along a broad, flat and polished surface at the tip. Correct any deficiencies using a sharpening stone. Time invested here will be duly rewarded.
 2. Newly eclosed flies should be collected and aged on fresh yeast paste for 3–5 days at 25 °C prior to dissection. Typically, 6–8 individual female flies are processed at a time.
 3. Place adults on fly pad. Cleanly remove wings and legs by pinching them off very close to the body with forceps.
 4. Next, grasp each fly by the proboscis and transfer to a watch glass filled with 95% ethanol, submerging completely for 30 s. This brief dehydration step facilitates wetting and removal of air bubbles trapped among adult bristles.
 5. Now, grasp each fly by the proboscis and transfer to a second watch glass filled with 1× PBS, submerging completely. Allow the flies to wash for 30 s in 1× PBS, diluting away the ethanol.
 6. Transfer the flies to a sylgard dissection dish containing 1× PBS, again by grasping the proboscis. The samples should now be resting at the bottom of the dish. If not, remove any visible air bubbles with forceps.
 7. Place a folded, PBS moistened kimwipe on the stage next to the sylgard plate. Each piece of tissue removed during the dissection will be deposited here. This keeps the buffer clean and prevents bits of tissue from re-adhering to the GI tract.

2.2. Dissection

The goal of this procedure is to obtain an intact GI tract from the adult fruit fly. Conceptually, this method is more accurately understood as the systematic removal of all non-GI tissue, rather than plucking the GI tract from the fly's belly. This can be achieved in five steps. Forceps are used to remove tissue along the paths indicated by red dashed lines (Fig. 2), as described below. Overall, the dissection begins at the abdomen and proceeds anteriorly to the head. The procedure is complete once the entire midgut and hindgut have

been isolated. At no point in the process should the midgut be touched directly with the forceps. Samples damaged during dissection do not perform well during subsequent processing and should be immediately discarded.

1. When a fly has been successfully prepared for dissection, all that will remain is the head, thorax and abdomen (Fig. 2A). Securely fasten the fly to a sylgard plate by placing a single minuten pin through the center of the head, while the fly is resting with its dorsal side down. Looking through the dissecting microscope the sample should be oriented with the head at the top of the field, and the abdomen at the bottom, closest to you.
2. The dissection begins at the posterior of the ventral abdomen (Fig. 2B and C). Using a pair of forceps, grasp the epithelium superficially to avoid damaging any internal organs. Next, initiate a small tear. Strip away the tissue by pulling anteriorly, first along one side of the abdomen at the margin of the pigmented cuticle, then along the other. Once complete, the contents of the abdomen should now be visible and resting within the otherwise intact abdominal cuticle.
3. Second, create a small crescent shaped tear in the abdominal cuticle separating the 8th and 9th tergites from the remainder of the cuticle (Fig. 2B and C). This cuticular fragment will contain the termini of both the GI and reproductive tracts. Use this patch of cuticle to lift the organs out and away from the remainder of the abdomen. As the GI tract is lifted out, progressively disrupt any of the fine silvery tracheal strands that bind the viscera to the cuticle using forceps.
4. Third, disconnect the abdominal cuticle at the narrow waist where it contacts the thorax (Fig. 2B and C). The contents of the abdomen should now be floating freely in solution. Finally, separate the GI tract from any remaining tissue. This will include the ovaries and reproductive tract, which in well-fed females can be easily coaxed away by gently tugging on the ovipositor.
5. The fourth step entails removing the muscle and cuticle of the thorax surrounding the GI tract (Fig. 2B and C). Exercise caution here, as many dissections fail at this stage. Begin separating the thoracic cuticle in the posterior, working anteriorly between the remaining stumps of cuticle where the legs were attached. Once you reach the anterior most region of the thorax it is necessary to tear the cuticular “collar”, through which the foregut passes into the head. This action will allow the thoracic muscle and cuticle to be completely removed from around the GI tract.
6. All that remains is to detach the thin foregut from the head just anterior to the cardia (Fig. 2B and C).
7. It is now appropriate to trim the sample. First, insert the tine from one of your forceps under the cardia, and then slide it posteriorly until the adhesions holding the crop and salivary glands to the midgut are detached. Next, strip away the wishbone shaped salivary glands. It is also advisable to free both the anterior-projecting and posterior-projecting pairs of malpighian tubules from their superficial points of attachment with the gut, again by gently tugging. At this point, the sample should now resemble the image shown in Fig. 1.

8. *Note:* Fully complete each step of the procedure before moving onto the next. By adhering to and perfecting each step you will gradually build speed and consistency in your dissection. When 8 samples can be dissected in 30 min, working competence is attained. On average, this requires performing a minimum of three independent dissections per week for about 3 months.
9. Following dissection, all GI samples are simultaneously transferred to fixative, as described below.

2.3. Fixation

2.3.1. Materials

- 16% EM grade formaldehyde (Polysciences #18814).
 - Phosphate buffered saline, 0.01 M (PBS: NaCl, 0.138 M; KCl, 0.0027 M; pH 7.4; Sigma #P3813).
 - 9 well glass depression plate (Corning #7220-85).
 - Deep plastic petri dish; 150 mm × 25 mm (Nunc #4014-12).
 - 4 °C incubator.
 - P1000 pipet.
 - Plastic pipet tips.
 - Fresh razor blades.
1. Many perfectly dissected gut samples, obtained at great cost, have been irretrievably stuck inside pipet tips during the transfer to fixation buffer. This problem can be greatly minimized by coating the inside of the transfer pipet tip, in advance, with either lipid or glycoprotein.
 2. *Note:* One of the most ready ways to coat a pipet tip is using saliva. Deposit some saliva in one of the watch glasses. Add a roughly equal volume of 1× PBS to the saliva. Using a fresh razor blade cut the end off of a P1000 plastic tip, at an oblique angle to increase tip diameter. Any jagged edges are certain to retain your gut samples. Draw the saliva/PBS solution up and down to coat the inside of the plastic tip. An alternative is to place at least 6–8 clean wandering third instar larvae that have been torn open to liberate the fat body into a 1.5 ml tube containing 1× PBS. To coat the pipet tip with lipid, vigorously draw the solution containing the larvae up and down. Be sure that the modified tip diameter is still narrow enough to atomize the larval tissues. The coating process is complete once larval tissue fragments no longer stick to the inner surface of the tip.
 3. Tissue is fixed in a cold 4% formaldehyde solution. Once the gut samples of interest have been obtained, remove a pre-chilled 9 well depression plate, 16% formaldehyde and 1× PBS from the 4 °C incubator. Assemble fixation buffer in each of the three wells along one side of the 9 well glass plate. First place 450 µl of 1× PBS in each well. Next, add 150 µl of 16% formaldehyde to each of the three

wells. Finally, mix the contents of each well completely by pipeting until the solution appears uniform under the dissecting microscope.

4. Working under the dissecting microscope with a coated pipet tip, carefully draw up the dissected samples all at once. Use the minimum volume of 1× PBS and hold samples just at the tip of the pipet to minimize the chance of sticking. Carefully deliver the samples to one of the corner wells containing fixation buffer. Once the samples have been expelled into the first well, lightly mix by puffing the buffer around the well. Serially transfer the guts into each of the next two wells using the same procedure, each time minimizing the volume of buffer transferred. This will ensure that tissue fixation occurs in undiluted 4% formaldehyde. Samples should now be resting on the bottom of the final glass well. Free any air bubbles associated with adherent trachea using forceps.
5. Remove fixation buffer from all but the final well containing the gut samples. Check to ensure that there is no fixative clinging to the edges of the glass plate. To create a humid chamber that will minimize evaporative loss, place 600 µl of 1× PBS in the middle well on the opposite side of the glass plate from the sample. Carefully, set glass plate inside deep plastic petri dish and cover. Place chamber on flat surface in 4 °C incubator and allow fixation to occur overnight without agitation (short protocol, 2 h).
6. *Note:* The extended fixation times recommended here preserve tissue features without compromising histological detection for almost all commonly used antigens.
7. The following morning remove fixation buffer from well, and any residual buffer that has accumulated along the edge of the plate while in transit. Working under the dissecting microscope, carefully exchange fixation buffer with four full volumes of cold 1× PBST in the well. Finally, fill the well with 600 µl of 1× PBST and wash for 8 h at 4 °C without agitation (short protocol, 2 h).

2.4. Immunostaining

2.4.1. Materials

- Phosphate buffered saline, 0.01 M (PBS: NaCl, 0.138 M; KCl, 0.0027 M; pH 7.4; Sigma #P3813).
 - 1× PBS + 0.1% Triton X-100 (PBST; Sigma–Aldrich #T8787).
 - Primary antibodies (e.g. DSHB).
 - Secondary antibodies (e.g. Alexa fluor conjugates 488, 568, 633).
 - P200.
 - Vectashield mounting media + DAPI (Vector Labs #H1200).
1. All solution exchanges are performed under the dissecting microscope. Remove 1× PBST wash from fixed samples and add primary antibodies diluted in 1× PBST. A

list of commonly used primary antisera and reporter lines marking gut cell types are shown in Table 1. Incubate samples in primary antibody overnight at 4 °C.

2. The next day, wash samples by exchanging three full volumes of 1× PBST in the well. Finally, add 1× PBST to fill half the well and incubate 8 h at 4 °C (short protocol, 2 h).
3. Remove wash and incubate with appropriate secondary antibodies diluted in 1× PBST overnight at 4 °C (short protocol, 3 h).
4. Wash samples by exchanging three full volumes of 1× PBST in the well. Finally, add 1× PBST half to fill the well and incubate 8 h at 4 °C (short protocol, 2 h).
5. Completely remove all PBST from 9 well plate and add 6 drops of Vectashield + DAPI from P200 tip. Any residual PBST left in the well will degrade DAPI stain. Mix thoroughly by stirring and allow samples to clear overnight at 4 °C (short protocol, 2 h).

2.5. Mounting

2.5.1. Materials

- Glass microscope slides (Fisherbrand #12-544-3).
- Cover glass; 22 × 30 mm (Fisherbrand #12-544-A).
- Permanent double-sided tape, 0.5 inch wide (3M #3136).
- Austerlitz insect pins; 000, black enameled (FST #26000).
- Nail polish.
- Microscope slide box (Fisherbrand #03-446).

Mounting midgut samples on microscope slides using tape spacers creates a mini chamber that helps to preserve the epithelial and luminal distinction in fixed samples during subsequent imaging (Fig. 3). In principle, multiple layers of tape can be used to create chambers of different sizes; in practice, one layer has proven to work well in a range of applications.

1. Prepare slide and cover glass by removing any dust particles with a kimwipe and 95% ethanol.
2. When the slide is completely dry, apply two parallel strips of double-sided tape perpendicular to the long axis of the slide. Break overhanging tape using the sharp edge of the slide so that it is flush with the glass. The tape should be spaced such that, when the cover glass is applied, 75% of the tape falls under the coverglass on each side.
3. Apply a drop of mounting media in the middle of the slide and spread it with an insect pin to create a thin aqueous film across the slide. Using a cut pipet tip transfer samples from 9 well depression plate to the glass slide. Take one final opportunity to inspect the sample removing any unnecessary tissue and random debris. Using a pair of insect pins arrange midguts so that they lie in their natural

coiled configuration, free of any torsional strain. Time invested here will be duly rewarded.

4. Once samples are in position, apply a line of fresh mounting media along the left side of the slide, parallel to the tape. Apply cover glass (Fig. 3). Gently run forceps over the surface of the cover glass where it contacts the tape on each side, so that it is firmly adherent. Observe that that positive pressure between the slide and cover glass distributes mounting media evenly throughout chamber. If any free air space remains, use a pipet to stream additional mounting media into the chamber at the open edge of slide. Allow sample to fully equilibrate and remove any excess mounting media from around the edge of the coverglass using a vacuum or blotting with the corner of a kimwipe. Finally, seal edges completely with nail polish. Samples can be imaged using compound or confocal microscopy (Fig. 4). For best results, image tissue immediately. Store slides in slide box at 4 °C.

3. Genetic mosaic analysis

Mosaic analysis entails the low frequency labeling of cells within a living organism to create a biological composite [4]. Inducible genetic marking is a means to permanently label cells with a histological tag (e.g. *GFP*, *Lac Z*). Thus, genetic mosaic analysis offers the advantage of being able to track a labeled cell, and any offspring it may produce, over time, in the native context of a tissue. In addition, some methods combine permanent cell labeling with the ability to manipulate gene function within the lineage. This ability to create small patches of mutant tissue or “clones” within an organism has proven to be of great value in the study of local interactions between adjacent cells. Such labeling methods are readily adapted for the analysis of cell lineages in the *Drosophila* GI tract. The whole mount immunostaining method described above can be used to visualize histological tags during lineage tracing studies.

3.1. Induction of genetic mosaics using mitotic recombination

Mitotic recombination between non-sister homologous chromosome arms can be experimentally induced in a dividing cell to give rise to permanently labeled daughter cells [5]. Commonly used methods currently employ the yeast Flpase enzyme, which catalyzes mitotic exchange between FRT sequences that have been engineered into the *Drosophila* genome [6–8]. The general protocol below has been used to follow the products of both wild type and mutant stem cell divisions in the adult gut using FRT/Flpase based labeling systems [9,10].

3.1.1. Materials

- 37 °C water bath.
- Adult *Drosophila*.
- Plastic food vials (Genesee #32-109BF).
- Cotton plugs (Genesee #49-102).
- 15 ml orange screwcap centrifuge tube (Corning #430052).

- Old blunt forceps.
- 1. Collect newly eclosed F1 offspring of the appropriate genotype and age for 3–5 days on yeast paste at 25 °C. For example, positively marked wild type cell lineages can be traced with either of the following genotypes: *hsflp; X-15-29/X-15-33* or *yw, hsflp, UAS-GFP; tubGal4, FRT^{82B}tubGal80/FRT^{82B}* (see [7,8] for detailed information on these strains).
- 2. Create an induction chamber by slicing a cotton plug in half with a razor blade and pressing it to the bottom of a standard fly vial with 15 ml tube. Moisten half plug with water, pour off any excess liquid, bump flies into chamber and seal with a second whole plug pressed flush to the top of the vial.
- 3. Transfer this vial to 37 °C water bath and submerge with a weight. Ensure that the level of the water bath falls between the top of the vial and the bottom of the plug.
- 4. Duration of heat pulse can be titrated depending on the degree of labeling desired. For example, a single 30 min pulse provides low frequency cell labeling of wild type lineages in the adult midgut. However, note that induction frequency can vary between chromosomes and with genetic background.
- 5. Allow flies to recover for 30 min on bench top, pry out cotton plug with blunt forceps, transfer flies to freshly yeasted vial and continue to culture at 25 °C until you are ready to fix and analyze the GI tract histologically or apply an environmental challenge.

4. Environmental challenge

Stem cell mediated regeneration of the gut epithelium occurs in response to environmental challenge [3]. In *Drosophila*, a growing list of experimentally imposed challenges has been shown to efficiently induce regeneration. Protocols to alter nutrient availability and administer enteric pathogens, or the heparan-like polysaccharide dextran sodium sulfate (DSS) have been established [11–13]. Two examples are discussed briefly below. In each case, a food vial containing either a customized media preparation or standard media laced with defined amounts of a specific agent is prepared. Flies are transferred into vials for defined times and exposed to agents by ingestion.

4.1. Nutrient availability

Deficiency or over abundance of dietary nutrients is a key physiological variable influencing development, homeostasis and disease processes. A powerful tool to dissect nutrient-host interactions is the availability of a chemically defined food (CDF) media, which consist largely of purified compounds. Such diets permit the systematic evaluation of individual dietary components or caloric density and combine powerfully with molecular genetic approaches. Great progress has been made in refining CDF for *Drosophila*, however complex additives such as agar or trace amounts of lecithin still remain; stage and genotype specific nutritional requirements are also evident [11,14]. Notwithstanding these caveats, current CDF formulations now offer the investigator a significantly expanded experimental platform from which to directly test the effects of individual dietary nutrients. Table 2A

shows the full list of ingredients necessary for the production of CDF formulated at a caloric density of 400 K-cal/L [11]. For detailed protocols and discussion the reader is referred to the following references [11,14].

4.2. Enteric pathogens

The naturally occurring Gram-negative bacterium *Pseudomonas entomophila* (*Pe*) disrupts the barrier epithelium and thus provides a powerful experimental tool to study the effects of enteric pathogens on gut homeostasis [15]. The mechanism by which *Pe* disrupts gut epithelial cells is not fully understood, but may involve the production of specific toxins. Spiking regular food vials with a concentrated solution of *Pe* induces stem cell based epithelial regeneration along the GI tract [10,12].

4.2.1. Materials

- 30 °C shaking incubator.
 - *Pseudomonas entomophila* (*Pe*) isolates.
 - Rifampicin plates; 1.5% agar; 2% LB; 1% non fat powdered milk (from autoclaved 10% wt/vol stock); 100 µg/ml rifampicin (from freshly thawed stock added just before pouring plates).
 - Rifampicin stock solution; 50 mg/ml in DMSO store at –20 °C.
 - LB.
 - 1 L glass flask.
 - 250 ml glass flask.
 - 5% sucrose solution.
 - Sterile pipet tips.
 - Sterile wooden applicators.
1. Preparation of high titer *Pe* solution for infection. Streak bacteria from –80 °C *Pe* stock onto rifampicin plate and culture for 2 days at 30 °C. Identify a protease positive *Pe* clone, which will appear clear due to the absence of milk. Pick a single colony using a sterile tip and initiate a 40 ml starter culture of LB + rifampicin in 250 ml flask. Grow for 8 h shaking at 30 °C. Next, use the small starter culture to initiate a second, larger culture. Dilute starter culture 1/16 in a final volume of 400 ml and culture for 18.5 h in 2 L flask. On the day of infection, pellet cells by centrifuging for 20 min at 2500g at 4 °C. Carefully remove supernatant and adjust OD by re-suspending pellet in 5% sucrose.
 2. A fresh batch of regular fly food lacking tegosept, a broad spectrum antifungal and antibiotic, should be prepared in advance and aliquoted, 3 mls media/ vial (Table 2B).

3. Add 0.5 ml of either 5% sucrose alone (control) or 5% sucrose + *Pe* (infection) to each vial. Using sterile wooden applicator, mix solution into food superficially, using only the minimum volume of food necessary to fully incorporate the solution.
4. Allow vial to set for 15–30 min. The vial will still be quite moist at this point.
5. Carefully introduce flies into vial for a defined period of exposure. During the infection vials should be left lying on their side.

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Fig. 1.

Anatomy of the adult gastrointestinal tract. A freshly dissected GI sample as seen under the dissecting microscope. Anterior to the left. Foregut (fg), crop (cr), cardia (c), midgut (mg), malpighian tubules (mt), pylorus (p), and hindgut (hg).

Fig. 2.

Procedure for dissecting the adult gastrointestinal tract. (A) An adult fruit fly prepared for dissection. Appendages are removed, the fly is washed, and a minuten pin is placed through the head securing the sample to a sylgard dish. (B and C) Removal of the adult gastrointestinal tract is a five-step process. Numbers indicate sequence in the process. Dashed red lines show dissection paths. Note, the thorax is not bisected and removed until step 4, as seen most clearly in B. Head (h), thorax (t), abdomen (a). (B) Lateral view. (C)

Ventral view. Original drawings by Miller, in *Biology of Drosophila*, were modified and reprinted with permissions.

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Fig. 3.
Chamber assembly for imaging whole mount gut samples on glass slide with spacers.

Fig. 4.

Cells of the adult midgut in whole mount. (A and B) The adult copper cell region (CCR); anterior (A), posterior (P). (A) Feeding with the pH indicator dye bromophenol blue reveals the acidic copper cell region with a pH < 3 (yellow). (B) The acid-secreting copper cells of the middle midgut (anti-Cut, white). DNA counterstain (DAPI, blue). (C and D) *esg-lacZ* expressing gastric stem cells (GSSC, white) are interspersed among copper cells (anti-Cut, red) and are located basally in the epithelial layer in close proximity to the surrounding visceral muscle (*24B>GFP*⁺, green). DNA counterstain (DAPI, blue). (C) Superficial section. (D) Cross-section. Scale bars: 200 μ m in B, 20 μ m in C and D. Reprinted in part with permissions.

Table 1

Commonly used molecular markers.

Marker	Cell type/feature	References
Delta (DI)	Intestinal stem cell	[16]
Sanpodo (Spdo)	Intestinal stem cell	[17]
<i>esg-gal 4</i>	Intestinal stem cell, gastric stem cell and enteroblast	[10,18]
<i>10xstat-gfp</i>	Intestinal stem cell and enteroblast	[12,20]
<i>Su(H)GBE-lac Z</i>	Enteroblast	[18]
Prospero (Pros)	Enteroendocrine	[18,19]
POU domain protein 1 (Pdm-1)	Enterocyte	[20]
<i>Myo1A-gal 4</i>	Enterocyte	[12]
Cut (Ct)	Copper cell	[10]
Labial (Lab)	Copper, interstitial cells	[10]
<i>dve-lac Z</i>	Copper, interstitial, large flat cells	[10]
<i>vkg>gfp</i>	Basement membrane	[20]
<i>24B-gal 4</i>	Visceral muscle	[21]
<i>vn-lac Z</i>	Visceral muscle	[21]

Table 2

A. Recipe for 400 K-cal/Liter chemically defined food (CDF^{400K}).			
Ingredients	g/L	Ingredients	g/L
Amino acids	19.61	Vitamins, minerals, and nucleic acids	3.20
L-arginine HCl	1.67	Vitamin B12 (0.1% in mannitol)	0.01880
L-histidine HCl-H ₂ O	0.47	Biotin	0.00002
L-isoleucine	0.81	p-Aminobenzoic acid	0.00200
L-leucine	1.32	Inositol	0.04200
L-lysine HCl	2.78	Niacin	0.01000
L-methionine	0.58	Calcium pantothenate	0.00599
L-phenylalanine	0.94	Folic acid	0.00599
L-threonine	0.90	Pyridoxine HCl	0.00300
L-tryptophan	0.74	Riboflavin	0.00241
L-valine	1.28	Thiamin HCl	0.00151
L-alanine	1.11	Choline bitartrate	0.03600
L-asparagine	0.53	Vitamin A palmitate (500,000 IU/g)	0.00270
L-aspartic acid	0.53	Vitamin E, DL-alpha tocopheryl acetate	
L-cystine	0.43	(500 IU/g)	0.03300
L-glutamic acid	1.20	Vitamin D3, cholecalciferol (500,000 IU/g)	0.00067
L-glutamine	1.20	Vitamin K, MSB complex	0.00051
Glycine	0.43	Zinc carbonate	0.01820
L-proline	0.90	Cupric carbonate	0.00850
L-serine	0.98	Chromium potassium sulfate, dodecahydrate	0.00540
L-tyrosine	0.81	Potassium phosphate, dibasic	0.60598
		Potassium phosphate, monobasic	0.60598
Carbohydrates	78.43	Calcium chloride	0.01291
Sucrose	63.68	Ferrous sulfate, heptahydrate	0.01291
Glucose	5.93	magnesium sulfate, heptahydrate	0.24599
Lactose	4.92	Manganese sulfate, monohydrate	0.00979
Trehalose	3.91	Sodium chloride	0.01291
		RNA	0.99991
Lipids	0.87	DNA	0.49996
Cholesterol	0.08		
Lecithin	0.79	Agarose	10.00
B. Recipe for tegosept free regular food (RF).			
Ingredients	g/L		
Yeast extract	50.00		
Yellow cornmeal	80.00		
Dextrose	50.00		
Agar	10.00		

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