Gastrointestinal motility is regulated by a variety of environmental factors including gut microbes and metabolites. The ability to interrogate mouse models of gut motility has enabled elucidation of these relationships. Here we describe integration of the red carmine dye and fluorescence isothiocyanate-dextran marker-based assays for characterizing gut transit with spatial resolution, along with an optional intracolonic infusion protocol for studying the effects of metabolites on colonic transit. These protocols can be adapted for use in gnotobiotic and conventional mouse models.
Protocol
Marker-based assays for studying gut transit in gnotobiotic and conventional mouse models

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https://doi.org/10.1016/j.xpro.2021.100938

SUMMARY
Gastrointestinal motility is regulated by a variety of environmental factors including gut microbes and metabolites. The ability to interrogate mouse models of gut motility has enabled elucidation of these relationships. Here we describe integration of the red carmine dye and fluorescence isothiocyanate-dextran marker-based assays for characterizing gut transit with spatial resolution, along with an optional intracolonic infusion protocol for studying the effects of metabolites on colonic transit. These protocols can be adapted for use in gnotobiotic and conventional mouse models.

For complete details on the use and execution of this protocol, please refer to Li et al. (2021).

BEFORE YOU BEGIN
The protocol below describes steps for studying gut transit in gnotobiotic or conventional mouse models. For guidance on working with gnotobiotic mice, please see (Zucoloto et al., 2021). We have adopted the commonly used red carmine dye assay to include intra-colonic administration of metabolites to test effects on gut transit times. We combine this approach with an assay utilizing the FITC-dextran fluorophore to spatially resolve whole-gut transit.

Prepare reagents

© Timing: 1–4 h

1. Prepare the necessary reagents described below. 200 μL per mouse of 6% carmine or 5 mg/mL FITC-dextran are administered for these assays. We recommend estimating the total volume needed per assay and preparing 10% more reagent than required.

△ CRITICAL: Sterilize reagents prior to use. We use 0.2 μm pore-diameter sterile filters for FITC-dextran, and we autoclave methylcellulose and carmine.

a. Prepare 0.5% methylcellulose solution. Using a hot plate, glass beaker, and magnetic stir bar, heat ~1/3 total final water volume to 80°C with agitation and add the methylcellulose powder.
Once particles are dissolved, add the remaining volume of water and cool solution to 0°C–5°C for 20–40 min. Store at 4°C until use.

b. Prepare 6% carmine solution. Add carmine to a 0.5% methylcellulose solution and heat with a magnetic stir bar until the carmine dissolves. Autoclave for 30 min at 122.8°C and 23.8 pounds per square inch. Store at 4°C until use.

c. Prepare FITC-dextran stock solution (20 mg/mL). We use 70,000 MW FITC-dextran as adapted from (Samuel et al., 2008). Allow FITC-dextran to dissolve in 1x PBS by wrapping in foil, vortexing, and placing at 4°C for 4 h. Dilute 250 μL stock solution with 750 μL PBS to obtain 5 mg/mL FITC-dextran solution. Filter-sterilize, wrap in aluminum foil, and store at −20°C until use.

Note: Methylcellulose and carmine are stable at 4°C for 1 year and to light; we recommend preparing carmine fresh for each experiment. FITC-dextran, which is light- and temperature-sensitive, should be wrapped in foil and stored at −20°C (stable for 6 months) or 4°C (stable for 3–4 weeks) prior to use.

d. Prepare 4 mM solutions of the bile acids to test. Our practice is to prepare solutions within 24 h of use and store at 4°C if needed. Dissolve 20 mg of bile acid in 5 mL of recommended solvent using a beaker and magnetic stir bar. Taurocholic acid and deoxycholic acid can both be dissolved in water or PBS. We recommend using PBS to account for dilution of other bile acids. Lithocholic acid should be dissolved in DMSO. Cholic acid and β-muricholic acid must first be dissolved in 1 mL of DMSO, then diluted with 4 mL PBS.

**KEY RESOURCES TABLE**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Chemicals, peptides, and recombinant proteins** | | |
| Methylcellulose | Sigma-Aldrich | M0512 |
| Carmine powder | Sigma-Aldrich | C1022 |
| Fluorescein isothiocyanate-dextran (FITC-dextran; 70,000 MW) | Sigma-Aldrich | 46945 |
| SBI-115 | MedChemExpress | HY-111534 |
| Phosphate buffered saline tablets (PBS) | Fisher-Scientific | BP2944100 |
| Deoxycholic acid | Sigma-Aldrich | 30960 |
| Lithocholic acid | Sigma-Aldrich | L6250 |
| Cholic acid | Sigma-Aldrich | C1129 |
| β-muricholic acid | Sigma-Aldrich | SML2372 |
| Taurocholic acid | Sigma-Aldrich | T4009 |
| Dimethyl Sulfoxide (DMSO) | Fisher-Scientific | D128-1 |

**Deposited data**

- Examples of data generated and scripts used for data analysis: Li et al. (2021) [https://github.com/DeyLab/Li_Koester_Lachance_et_al_/iScience_2021/](https://github.com/DeyLab/Li_Koester_Lachance_et_al_/iScience_2021/)

**Experimental models: Organisms/strains**

- Mouse: Wild-type Swiss-Webster, male or female, age 6+ weeks [http://www.uwgonobiotics.org/](http://www.uwgonobiotics.org/) N/A

**Other**

- Spectrofluorometer: BioTek Synergy 2
- Fisherbrand Isotemp Hot Plate Stirrer: Fisher Scientific SP88854200
- Fisherbrand Polygon Stir Bars: Fisher Scientific 1451174
- Mouse restrainer: IBI Scientific MH-100
- Feeding needle: Braintree Scientific, Inc. N-PK-020
- 1 mL syringe: BD Vacutainer Labware Medical 14823434
- 5 mL syringe: BD Vacutainer Labware Medical 1482945

(Continued on next page)
**MATERIALS AND EQUIPMENT**

**Methylcellulose**

| Reagent               | Final concentration | Amount (for 100 mL) |
|-----------------------|---------------------|--------------------|
| Methylcellulose       | 5 g/L               | 0.5 g              |
| ddH<sub>2</sub>O       | n/a                 | 100 mL             |
| **Total**             | **0.5% methylcellulose** | **100 mL**         |

**Red carmine dye**

| Reagent               | Final concentration | Amount (for 20 mL) |
|-----------------------|---------------------|--------------------|
| 0.5% methylcellulose  | 5 g/L               | 20 mL              |
| Carmine               | 60 g/L              | 1.2 g              |
| **Total**             | **6% carmine**      | **20 mL**          |

**FITC-dextran**

| Reagent                | Final concentration | Amount (for 1 mL) |
|------------------------|---------------------|------------------|
| FITC-dextran           | 20 g/L              | 20 mg             |
| 1x PBS                 | n/a                 | 1 mL              |
| **Total**              | **20 mg/mL FITC-dextran** | **1 mL**          |

**STEP-BY-STEP METHOD DETAILS**

*Note:* These experiments can be conducted on conventional or gnotobiotic mice (germ-free or colonized). For guidance on working with gnotobiotic mice, please see (Zucoloto et al., 2021).

*Note:* Careful consideration of sample size accounting for covariates of interest is recommended. To sufficiently power your study, consider performing a pilot study to determine effect sizes.

**Carmine red dye assay—Part 1**

*Timing: 2–8 h (conventional mice have shorter transit times, while germ-free mice have longer transit times)*

Whole gut transit times can be quantified by administering the 6% carmine solution via oral gavage using a 1 mL syringe attached to a feeding needle and recording time to passage in the fecal stream.
If working in the gnotobiotic setting, additional sterilization techniques should be followed during the carmine gavage and fecal observation (e.g., sterilization of the fume hood, cage, equipment, and reagents), and a sterile gown and gloves should be worn by research staff.

1. Gavage each mouse with 150 μL of 6% carmine solution and record the time of administration.

   Note: It may be helpful to mark the tail of each mouse with a sequential number using a marker for easy identification (e.g., I, II, III, etc).

   ◆ Pause point: Given that whole gut transit times are typically ≥2 h after carmine gavage, a 2-h pause can be instituted prior to the first time point at which fecal pellets are examined.

2. Obtain fresh fecal pellets.
   a. Transfer each mouse individually into a sterile beaker or sterile plastic container (e.g., empty 1000 μL pipette tip box cleaned with 70% w/v ethanol) until a fecal pellet is produced.
   b. Immediately after the production of a fecal pellet, return the mouse to its cage.

   Note: It is helpful to cover the container with a weighted lid to minimize the risk of a mouse escaping. If no fecal pellets are seen within 5 min, it is reasonable to return the mouse to its cage and repeat this step in 5–10 min.

3. Assess fecal pellets for passage of red dye.
   a. Record all times at which assessments are made, including documentation of whether fecal pellets are produced and whether red carmine dye is seen at those times.
   b. If the pellet contains NO red color, continue to repeat the observation every 20–30 min until a red pellet is seen. If the pellet clearly contains red dye, no further observations are required.

   Optional: Smear fecal pellets on white tissue if there is a question of whether carmine passed, as this permits easier visualization.

4. Whole gut transit time is calculated as the time interval between the gavage and the first observed red fecal pellet. A fecal pellet that is partially red may constitute a red pellet if the red color is carefully examined and convincingly felt to represent carmine dye.

△ CRITICAL: If studying gnotobiotic mice, then the beaker or plastic container used to observe mice must first be sterilized (e.g., via autoclave). Containers should NOT be shared by mice harboring different microbiota.

Note: We grant mice ad libitum access to food and water during the red carmine assay as it typically takes greater than 2 h and up to 8 h (particularly in gnotobiotic mice) to complete. If necessary, researchers can consider a standardized period of fasting, but the impacts of prolonged fasting on mouse health must be carefully considered.

Note: We do NOT squeeze or apply pressure to the mouse abdomen to stimulate passage of fecal pellets, as stress also influences gut transit.

Note: Typically, the time to clearance is not recorded for practical reasons, as it can take >1 day; however, this could be considered with extended observation or through video monitoring of cages. “Time to clearance” is defined as the amount of time it takes for carmine to be passed in its entirety (i.e., the duration required for fecal pellets to no longer be red).

Note: Transit times are sensitive to time of carmine gavage and the light/dark cycle; therefore, if intending to compare between cohorts, it is essential to standardize one’s protocol. We
administer carmine between 8:00 and 8:30 AM local time. If needed, limit the number of mice that you are testing on a given day.

**Intra-colonic administration of bile acids (or other metabolites or treatments of interest)—Part 2 (optional)**

- **Timing:** 0.5–1.5 h

Treatments of interest can be administered via intra-colonic infusion to characterize effects on the distal colon. Intra-colonic infusions are paired with the carmine red dye assay (Part 1) to quantify the effects of infusions on whole gut transit times. We have performed this in conventional mice only. Gnotobiotic mice will require additional sterility precautions not described here. Although we have not used it as such, this could potentially be paired with a rectal bead expulsion assay (Soret et al., 2010).

△ CRITICAL: Evaluate the safety and potential toxicity of administered substances prior to administration. Safety considerations should be given for both the mice and personnel administering the metabolites. All substances should be approved by the biosafety committee and animal ethics committee prior to use.

△ CRITICAL: The timing of when to administer the metabolites of interest depends on how long the metabolites take to exert a physiologic effect and their half-life.

△ CRITICAL: Select the appropriate controls for your experimental infusions (e.g. vehicle-only control).

△ CRITICAL: Since the carmine red dye assay is sensitive to the time of day at which it is performed and the light/dark cycle, it is essential that intra-colonic infusions and carmine gavages are performed as uniformly as possible (i.e., at the same times and intervals) across treatment groups.

5. Attach a feeding needle to a 1 mL syringe and aspirate 400 μL of infusion media. To minimize discomfort, apply a layer of petroleum jelly to the needle with a sterile Q-tip. Ensure the needle tip is covered.

6. Carefully remove one mouse from the cage and place into the restrainer (Figure 1).  
   
   **Note:** Permitting time for the mouse to first pass fecal pellets will minimize rectal leakage when administering the infusion.

7. Apply petroleum jelly to the anus of mouse with a sterile Q-tip and slowly insert feeding needle 5 mm into the rectum. The tip of the needle is 5 mm; thus, stop pushing when the tip is fully inserted.

8. Slowly infuse 400 μL of infusion media into the rectum and carefully remove the needle. Maintain the mouse in Trendelenburg position for several seconds.

   **Note:** Perform this procedure quickly to avoid the mouse from moving or turning around in the restrainer.

9. Place the mouse into a clean cage for recovery. Monitor the mouse’s condition according to institutional safety operating procedures.

10. Repeat steps 5–9 for remaining mice.
Critical: Call veterinary services, such as the Animal Welfare and Compliance Officer or Veterinarian, if any bleeding from the anus is observed.

Optional: Repeat carmine and intra-colonic infusions as needed to characterize the effect of different metabolites on distal colonic transit. For studying additional treatments, we advise intervals of ≥72 h between intra-colonic infusions.

**FITC-dextran assay—Part 3**

## Timing: 3–5 h

Characterization of intestinal distribution of an orally delivered fluorophore permits spatial resolution of gut transit. This step must be done after measuring transit times with carmine as it requires euthanization. Auto-fluorescence can be impacted by diet; therefore, it is important to perform a pilot experiment characterizing the impact of the mouse chow being used by quantifying fluorescence in mice not receiving FITC-dextran. Gnotobiotic mice will require additional sterilization precautions during the FITC gavage such as sterilization of the fume hood, cage, equipment, and reagents; additionally, a sterile gown and gloves should be worn by research staff.

11. Gavage each mouse with 200 μL of 5 mg/mL FITC-dextran.

**Note:** It may be helpful to mark the tail of each mouse with a sequential number using a marker for easy identification (e.g. I, II, III, etc).

**Pause point:** Given that there needs to be sufficient time to conduct the flushes described in step 15, we recommend an interval of 20–30 min between FITC-dextran gavages (i.e., between mice).

**Note:** It is helpful to set up the dissection equipment during one of the pause points. Supplies will vary depending on what samples are collected, but the following are required for FITC flushes: a dissection board, two 100 mL beakers of cold PBS for motility inhibition and FITC flushing, a 5 mL syringe, feeding needle, and twelve 5 mL Eppendorf tubes per mouse (1 per gut segment) labelled 1–12.
12. Euthanize mice one at a time using your institutionally approved protocol.

13. Remove the gastrointestinal tract from stomach to the rectum and place in ice cold 1× PBS for 30 s to inhibit motility.

14. Using a razor or scalpel, divide the entire GI tract into 12 segments, according to the following list (Figure 2):
   a. Stomach.
   b. Small intestine (divided into 8 segments, numbered from proximal to distal).
   c. Colon (divided into cecum, proximal, and distal colon).

   Note: We numbered the segments from proximal to distal with the stomach as segment 1 and the distal colon as 12. Any numbering system will work, but the researcher should plan this out ahead of time.

15. Flush each gut segment:
   a. First draw 2 mL of ice cold 1× PBS into a 5 mL syringe attached to a feeding needle.
   b. Then, using forceps, pick up each gut segment and align directly above its corresponding 5 mL Eppendorf tube (e.g., the distal colon contents will be flushed into tube #12).
   c. Finally, insert feeding needle into one end of the gut segment and slowly flush contents with PBS into the 5 mL Eppendorf tube. Withdraw feeding needle when flush is complete.
   d. Close and shake Eppendorf tube to mix contents.
   e. Repeat for each of the 12 gut segments. For more guidance, see Figure 3.

16. Once all segments are flushed, immediately store samples in their proper conditions. Wrap Eppendorf tubes in aluminum foil, and store at 4°C or on ice until FITC is quantified.

△ CRITICAL: Storage conditions are important to preserve fluorescence. When flushes are complete, wrap Eppendorf tubes in aluminum foil to limit the exposure to light, and store at 4°C or on ice.

Figure 2. Dissection of mouse gastrointestinal tracts
(A) Example of a conventional mouse GI tract with each gut segment cut and labelled.
(B) Example of germ-free mouse GI tract, notable for enlarged cecum.
Sl, small intestine, numbers indicate segment: 1 = most proximal, 8 = most distal.
Pause point: Depending on the time between FITC gavages and the experience level of the researcher, there may be a 10–20-min gap between flushes. This is a good time to clean up the dissection board, store samples in the proper conditions, and prepare for the next mouse.

17. Finish flushing procedures for all remaining mice before processing samples.

△ CRITICAL: Fluorescence in flush contents should be quantified immediately after completing all flushes to ensure accurate detection of FITC.

18. Process samples for fluorescence quantitation:
   a. Transfer 110 μL of FITC flush contents into an opaque black 96-well plate. If using the suggested plate organization shown in Table 1, flushes should be aliquoted into columns 1 and 7.
   b. Perform a 1:10 dilution series as per Table 1:
      i. Aliquot 90 μL of 1X PBS into each empty well of columns 2–6 and 8–12.
      ii. Then perform a serial dilution, starting with 10 μL in columns 1 and 7, and stopping at columns 6 and 12, respectively. This is best performed using a multi-channel pipette.
      iii. Remove 10 μL from 1:100,000 wells, columns 6 and 12.
   19. Quantitate fluorescent signal using a plate reader. We use a BioTek Synergy 2 (excitation at 485/20 nm, emission at 530/20 nm).

   Note: Plate replicates are recommended. We typically perform triplicates.

   Note: To establish the limits of detection for FITC, we made 10-fold dilution series using different starting concentrations of FITC-dextran (e.g. 1 mg/mL, 0.1 mg/mL, 0.01 mg/mL.)
We found that measurements from a ten-fold dilution series starting at 0.05 mg/mL were linear (R > 0.99, R² > 0.99) and captured the range of values we observed in samples from GI tract flushes.

**EXPECTED OUTCOMES**

This protocol for measuring intestinal motility allows for the quantification of the whole gut transit time as well as the spatial resolution of intestinal transit. Whole gut transit times from the red carmine assay reflect the foremost tip of the digesta traversing through the gastrointestinal tract, while whole gut transit times from the FITC-dextran assay enables characterization of the distribution of the digesta through the gastrointestinal tract. Whole gut transit times for different cohorts can be plotted and compared such as in Figure 2E (Li et al., 2021). Spatial resolution of different cohorts can be plotted as a smoothed curve or as geometric means like Figure 2D (Li et al., 2021).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

FITC readings can be used in several ways. Transforming readouts into proportions of total FITC or absolute concentrations present in each gut segment or segment can be helpful in characterizing the spatial distribution of FITC using a smoothed curve. The total amount of FITC present throughout the gut can indicate recovery during the flushing procedure (if performed before FITC has fully transited through the gut i.e., in less time than the whole gut transit time) or how much might have already been passed in a fecal pellet. To compare FITC distributions between groups, we used the geometric mean (g) of fluorescence as a summary statistic, which we calculated using the following equation:

\[ g = \prod_{i=1}^{12} (i \times \text{FITC}_i) \]

For examples of data and R scripts that you can adopt in performing these calculations and creating smoothed curves, we refer you to the following links:

[https://github.com/DeyLab/Li_Koester_Lachance_et_al_iScience_2021/blob/main/Table_S3_For_GitHub_v210415.txt](https://github.com/DeyLab/Li_Koester_Lachance_et_al_iScience_2021/blob/main/Table_S3_For_GitHub_v210415.txt)

[https://github.com/DeyLab/Li_Koester_Lachance_et_al_iScience_2021/blob/main/geometric_means_calculation.R](https://github.com/DeyLab/Li_Koester_Lachance_et_al_iScience_2021/blob/main/geometric_means_calculation.R)

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Table 1. Example of FITC plate organization

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|----|----|----|
| A | Stomach | Stomach | Stomach | Stomach | Stomach | SI-8 | SI-8 | SI-8 | SI-8 | SI-8 | SI-8 |
| B | SI-1 | SI-1 | SI-1 | SI-1 | Cecum | Cecum | Cecum | Cecum | Cecum | Cecum | Cecum |
| C | SI-2 | SI-2 | SI-2 | SI-2 | PC | PC | PC | PC | PC | PC | PC |
| D | SI-3 | SI-3 | SI-3 | SI-3 | DC | DC | DC | DC | DC | DC | DC |
| E | SI-4 | SI-4 | SI-4 | SI-4 | SI-4 | 1x | PBS | PBS | PBS | PBS | PBS |
| F | SI-5 | SI-5 | SI-5 | SI-5 | SI-5 | 1x | PBS | PBS | PBS | PBS | PBS |
| G | SI-6 | SI-6 | SI-6 | SI-6 | SI-6 | 0.05 mg/mL | FITC | FITC | FITC | FITC | FITC |
| H | SI-7 | SI-7 | SI-7 | SI-7 | SI-7 | 0.05 mg/mL | FITC | FITC | FITC | FITC | FITC |
| DC, distal colon; PC, proximal colon; SI, small intestine, numbers indicate segment: 1 = most proximal, 8 = most distal. Ratios represent the serial dilution. 0.05 mg/mL FITC is positive control to quantify absolute amount of FIT in each intestinal segment. 1xPBS is negative control.
LIMITATIONS
A strength of the carmine red dye assay is that it can be administered repeatedly; its primary limitation is that it does not yield spatial resolution — only whole gut transit times. In contrast, the FITC-dextran assay can only be used once, as it entails euthanasia; however, it offers spatial resolution.

Studying the effect of metabolites on other parts of the intestine using the protocols described here would be challenging due to difficulties infusing metabolites into the small intestine or proximal colon. A researcher might consider anterograde gavage into the stomach, but absorption (e.g., bile acid absorption in the small intestine) and modification may confound the results.

Another consideration is translocation of FITC-dextran out of the gut. Indeed, FITC can be used for measuring gut permeability, and the gut microbiome impacts permeability; therefore, microbiome-driven differences in translocation of FITC-dextran from the gut lumen into the blood stream could confound its use as a gut motility assay.

TROUBLESHOOTING

Problem 1
Fluorescence too high or too low to be quantitated by the plate reader (step 19).

Potential solution
Establish a FITC standard curve for the plate reader as this allows for quantitation of FITC concentrations in samples with unknown concentrations. Establishing limits of detection is important because upper and lower limits are often instrument-specific and measurements will vary across plate readers from different manufacturers, even in samples with identical FITC concentrations.

We made 10-fold dilution series using different starting concentrations of FITC-dextran (e.g., 1 mg/mL, 0.1 mg/mL, 0.01 mg/mL vs. 0.5 mg/mL, 0.05 mg/mL, 0.05 mg/mL). We found that measurements from a ten-fold dilution series starting at 0.05 mg/mL were linear (R > 0.99, R² > 0.99) and captured the range of values we observed in samples from GI tract flushes.

Problem 2
Unexpectedly low fluorescence readouts in the FITC-dextran assay (step 19).

Potential solution
Increase the time interval between the carmine and FITC-dextran assays. We have found that carmine decreases the measured fluorescence when added directly to wells containing known concentrations of FITC-dextran. As reported in our recent paper (Li et al., 2021), we found that a 72-h interval between assays was sufficient in our model to prevent significant interference by carmine.

Problem 3
Blockage of pipette tips during FITC sample processing due to the larger particles of the flushed intestinal material (step 18).

Potential solution
The particulate matter in flush contents is usually quite large and will fully settle to the bottom of the tube after approximately 5 min. If tips get blocked, we recommend using a wide bore pipette tip when performing the dilutions.

Problem 4
Outliers in FITC quantification (step 19).
Potential solution
To get a sense for acceptable variation between triplicates, we first recommend assessing technical variation attributable to your instrument through repeat measurements of a known concentration of FITC. To minimize variability in measurements of your samples (i.e., the FITC flushes), we recommend gently shaking or pipetting up and down before transferring samples to the 96-well plate. In our experience, as reported in Li et al., (2021), we found that data from triplicates were largely concordant. We observed just a single outlier in 1 set of triplicates (overall <0.5% of cases); this outlier was >5 standard deviations removed from the other replicates. The precise threshold that you set should be based on careful consideration of your data.

Problem 5
Difficulty performing intra-colonic administration of metabolites (step 8).

Potential solution
It helps to practice intra-colonic administration using sedation, although we would recommend against sedation in experiments due to effects of sedation on gut motility.

Problem 6
Difficulty flushing intestinal segments (step 15).

Potential solution
FITC flushes are most easily performed through a two-person procedure in which person #1 uses forceps to initially stabilize and secure the gut segment over the 5 mL tube. After person #2 starts flushing PBS, person #1 will then use forceps to gently guide flushes down gut segments, thereby minimizing backflow and spillage. A third person can also be helpful in expediting the assay overall through management of the sample collection and storage.

RESOURCE AVAILABILITY
Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Neelendu Dey (ndey@fredhutch.org).

Materials availability
This study did not generate new unique reagents.

Data and code availability
Examples of data generated and scripts used for data analysis are available at https://github.com/DeyLab/Li_Koester_Lachance_et_al_iScience_2021/

ACKNOWLEDGMENTS
This work was supported by NIH grants (NIDDK K08 DK111941 and NCI Cancer Center Support Grant P30 CA015704 to N.D.), as well as pilot funds and start-up funds from the Fred Hutchinson Cancer Research Center (N.D.). Graphical abstract and figures created using images from BioRender.com.

AUTHOR CONTRIBUTIONS
Conceptualization, S.T.K., N.L., D.M.L., and N.D.; data curation, S.T.K., N.L., D.M.L., and N.D.; formal analysis, S.T.K., N.L., D.M.L., and N.D.; funding acquisition, N.D.; investigation, S.T.K., N.L., D.M.L., and N.D.; methodology, S.T.K., N.L., D.M.L., and N.D.; project administration, S.T.K., N.L., D.M.L., and N.D.; resources, N.D.; software, S.T.K., D.M.L., and N.D.; supervision, N.D.; validation, S.T.K., N.L., D.M.L., and N.D.; visualization, S.T.K. and N.D.; writing – original draft, S.T.K., N.L., D.M.L., and N.D.; writing – review & editing, S.T.K., N.L., D.M.L., and N.D.
DECLARATION OF INTERESTS
The authors declare no competing interests.

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