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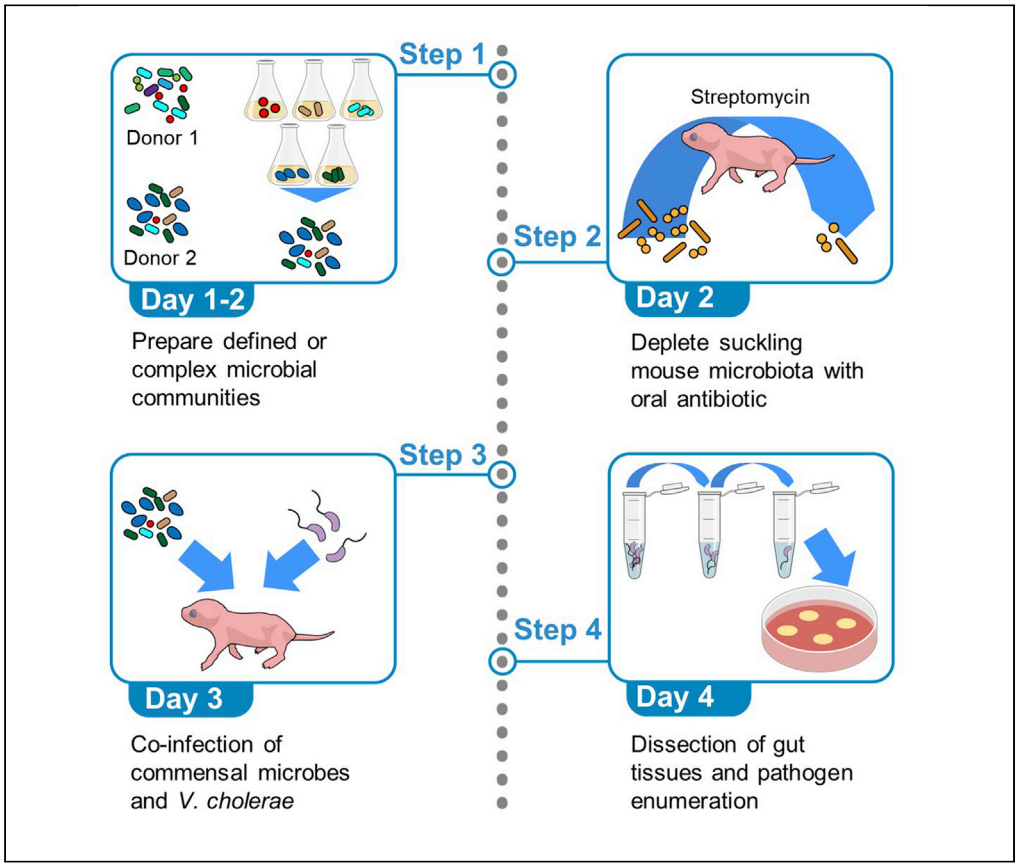
Protocol for Microbiome Transplantation in Suckling Mice during *Vibrio cholerae* Infection to Study Commensal-Pathogen Interactions



The gut microbiome plays an important role in the exclusion of pathogens and, thus, infection outcomes. Microbiome-pathogen interaction studies are complicated by a lack of tractable animal models and differences in animal model versus human microbiomes. We have adapted the suckling mouse model of infection of the human pathogen *Vibrio cholerae* to clear murine microbes and establish human-associated gut microbes during infection. Our method allows for the easy examination of the contribution of different human microbial communities to enteropathogenesis.

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HIGHLIGHTS

A protocol for testing interactions of human microbiomes and *Vibrio cholerae*

Depletion of murine commensals allows for replacement with human gut bacteria

Detailed procedures for preparation of bacterial mixtures and gavage of suckling mice

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Protocol for Microbiome Transplantation in Suckling Mice during *Vibrio cholerae* Infection to Study Commensal-Pathogen Interactions

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SUMMARY

The gut microbiome plays an important role in the exclusion of pathogens and, thus, infection outcomes. Microbiome-pathogen interaction studies are complicated by a lack of tractable animal models and differences in animal model versus human microbiomes. We have adapted the suckling mouse model of infection of the human pathogen *Vibrio cholerae* to clear murine microbes and establish human-associated gut microbes during infection. Our method allows for the easy examination of the contribution of different human microbial communities to enteropathogenesis.

For complete details on the use and execution of this protocol, please refer to Alavi et al. (2020).

BEFORE YOU BEGIN

The gut microbiome participates in numerous host-related phenotypes, including susceptibility to pathogens. The substantial differences between human and native animal model microbiomes complicate studies of microbiome-pathogen interactions (Seedorf et al., 2014). Conventionally-reared animals without modification are highly refractory to *V. cholerae* colonization (Olivier et al., 2009), while existing germ-free animal models for cholera are expensive and difficult to breed and maintain. Here, we describe an easily accessible animal model for studying interactions between human gut microbiome and *V. cholerae* in the context of infection. We have adapted the popular suckling mouse model of *V. cholerae* infection (Klose, 2000) through pre-infection treatment with the antibiotic streptomycin to substantially reduce existing mouse microbes, followed by the establishment of human-associated gut microbes to examine microbiome-pathogen interactions during infection. Animals can be inoculated with complex human fecal specimens or with defined mixtures of pure bacterial cultures.

Option 1: Prepare Defined Communities of Bacteria for Gavage

Timing: 1–2 h, ~ 72 h incubation, 30 min next 3 days, ~48 h incubation, 1–2 h next 2 days

Note: Systems for generating anaerobic conditions may vary. We performed experiments using a Coy vinyl anaerobic chamber with internal incubator cabinet and atmosphere of 7% H_2, 20% CO_2, and balance N_2. The chamber employs a palladium catalyst reacting with H_2 to remove atmospheric oxygen by conversion to water. Typical O_2 concentrations are <10 ppm.

1. Ensure that there is sufficient deoxygenated media for experiment available. At least 3 days before the start of the experiment, place sterilized reagents, and autoclaved glass vials and crimp-top sealing caps with polyurethane septa, into anaerobic chamber with loose caps to allow for deoxygenation/pre-reduction.
2. Prepare media and reagents. Refer to "Materials and Equipment" for component list of LYH-BHI media. Add all components except hemin to a glass beaker. Stir at 52°C and add hemin until dissolved. When the solution is clear, sterilize by filtration through 0.22 μm filter. Filter-sterilize 50% vol/vol glycerol and place into anaerobic chamber.
3. Prepare glycerol stocks from pure culture of anaerobic human isolates. Streak to single colony of suitable agar growth medium and pick one isolated colony for inoculation into 5 mL liquid broth. For anaerobic strains, culture at 37°C in anaerobic chamber until late logarithmic phase (varies by species). Mix 1:1 vol/vol culture with filter-sterilized and deoxygenated 50% glycerol, mix by pipetting, and aliquot 300 μL in 2 mL glass vials with crimp-top caps with polyurethane septa. Place crimp-seal caps on vials with forceps sterilized with 70% vol/vol ethanol and crimp to seal. Cycle from anaerobic chamber and freeze immediately at −80°C.
4. To start cultures for colonization, thaw the glycerol stock cultures of the bacterial strains in anaerobic chamber at room temperature (20°C–22°C). Stocks of aerobic strains can be thawed outside the chamber. Remove culture by syringe from thawed glycerol stock into 5 mL LYH-BHI media. Incubate for 3 days at 37°C.

⚠ CRITICAL: If the strain is a strict anaerobe, incubate and prepare cultures and stocks under anaerobic conditions.

Note: Culture preparation for colonization studies starts 5 days before the start of the experiment. Preparation of complex human fecal samples can start on the same day of the start of the experiment.

5. After 3 days (2 days before the start of the experiment), dilute the cultures (1:50) in fresh LYH-BHI media. Incubate at 30°C for 48 h.
6. After 2 days (on the day of the experiment), measure the optical density (OD_{600}) of the culture. Use at least 100 μL of the culture for measurement.
7. Normalize cultures for density by OD_{600}. The equivalent of a total of 300 μL of 0.4 OD_{600} culture should be divided evenly across strains by community. For example, a gavage of a 10-member defined community will contain the equivalent number of cells in 30 μL of 0.4 OD_{600} culture of each member strain.
8. Mix the calculated amount for each strain together, centrifuge for 2 min at 9,000 × *g* to pellet. Resuspend combined cell pellet in fresh LYH-BHI media to a maximum of 50 μL per suckling mouse.

⚠ CRITICAL: Pelleting of anaerobic strains should be performed in an anaerobic chamber. For mixed anaerobic/aerobic strains, pellet aerobic strains in normal atmosphere, and transfer cell pellets to the anaerobic chamber to resuspend in fresh deoxygenated LYH-BHI media.

Note: Amount of LYH-BHI to resuspend cell pellets varies based on number of animals to be gavaged. Each mouse receives a maximum gavage volume of 50 μL. Mice not receiving *V. cholerae* at time of gavage can receive a community mixture of 50 μL. For co-infections, the cells of the community mixture should be resuspended in 25 μL final volume, with the remaining 25 μL being the *V. cholerae* inoculum, generally 1×10^4–1×10^5 CFU *V. cholerae* in LB media.

Note: More than one gavage may be required if the first is not successful. In this case, gavages can be attempted while the mouse is still slowed from first exposure to isoflurane. If the mouse

does not appear well or the color of the skin has changed, place back in the incubator and try to gavage again after 15–30 min.

9. Prepare *Vibrio cholerae* inoculum. One day before the start of colonization experiment, inoculate *V. cholerae* from glycerol stock in 3 mL LB media and incubate at 37°C for 18 h. On the day of the experiment dilute the culture (1:100 vol/vol) in fresh LB in 125 mL flask and incubate at 37°C for 3 h with 250 rpm agitation on shaking incubator until the OD_{600} reaches ~ 0.3. Dilute culture (1:10) in fresh LB. Pellet by centrifugation, and transfer cell pellet into anaerobic chamber and resuspend in 1 mL deoxygenated LYH-BHI. Dilute 1:1,000 of this for gavage. Each mouse receives 25 μL of *Vibrio cholerae* inoculum.

⚠ CRITICAL: When preparing gavages for a large number of mice, some of the inoculum will remain in the needle and syringe. To ensure that sufficient inoculum is available, consider preparing 2 times more inoculum than calculated as necessary for the number of mice to be gavaged.

Option 2: Prepare Complex Human Fecal Samples for Gavage

Timing: 4–5 h (after sample collection), 1–2 h on the day of the experiment

10. Human fecal samples are collected separately from each donor in specimen collection containers of choice (e.g., Fisherbrand Commode Specimen Collection System). After collection, stool samples can be processed immediately or frozen at −80°C (for up to 6 months) for later processing.

Note: Pooled samples would not be appropriate for the determination of inter-individual variation in pathogen-microbiome interactions.

11. To begin sample processing, transfer fecal specimens into anaerobic chamber on ice. Remove 2–3 cm piece with chisel sterilized with 70% vol/vol ethanol and weigh. Add 1:3 weight/volume of sterile deoxygenated PBS containing 0.1% wt/vol cysteine. Vortex for 5 min to resuspend.
12. After resuspending in PBS, pass sample through the sterile 70 μm cell strainer to remove large particulates.
13. Add sterile deoxygenated 50% weight/volume sterile glycerol to a final concentration of 25% vol/vol glycerol. Aliquot into 500 μL into 2 mL glass vial (large opening crimp-top). Sterilize tips of tweezers with 70% vol/vol ethanol and use to place 11 mm crimp-seal red rubber septa (cap). Take care to avoid contact with the interior of the cap. Crimp cap to seal. Freeze stocks immediately at −80°C.
14. Quantify the bacteria in each sample by quantitative real-time PCR measurement of bacteria 16S DNA. Normalize fecal slurries to be representative of 16S levels in 300 μL of OD_{600}=0.4 culture of defined community described above. Primers: 5′-CTCCTACGGGAGGCAGCAG-3′, 5′-TTACCGCGGCTGCTGGCAC-3′.

Note: For detailed protocol of quantitative real-time PCR, please refer to (Alavi et al., 2020).

15. On the day of the experiment, thaw sufficient prepared stocks in anaerobic chamber (volume requirements depend on number of animals; see step 7, Before You Begin for calculation). Centrifuge the calculated amount for 2 min at 9,000 × *g* to pellet. Resuspend cell pellet in fresh LYH-BHI media. LYH-BHI volume depends on the number of mice to be gavaged. See above after step 8.
16. If you have any aerobic strains, prepare as explained above, take the pellet to the anaerobic chamber and resuspend in calculated amount of fresh LYH-BHI or suitable media.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and Virus Strains		
Vibrio cholerae C6706 El Tor	Hsiao Lab stock	C6706
Biological Samples		
Human volunteer donor fecal sample	Alavi et al., 2020	N/A
Chemicals, Peptides, and Recombinant Proteins		
Isoflurane liquid inhalation 99.9% glass bottle 250 mL	Henry Schein	N/A
BHI broth (granulated)	Research Products International	Cat# B11000-1000.0
Yeast extract	Thermo Fischer Scientific	Cat# 210929
Hemin	MP Biomedicals	Cat# ICN19882010
Cellobiose	Fischer Scientific	Cat# AC108461000
Maltose	Millipore Sigma	Cat# M5885
Cysteine	Millipore Sigma	Cat# C6852
NaCl	Fischer Scientific	Cat# S271-10
Tryptone	Fischer Scientific	Cat# BP1421-500
Agar, powdered, Bacto-Style	Genesee Scientific	Cat# 20-274
PBS 10× powder concentrate	Fisher Bioreagents	Cat# BP665-1
Phenol:chloroform:isoamyl alcohol (25:24:1)	Fisher Scientific	Cat# BP1752I400
SDS	Fisher Scientific	Cat# O2674-25
Isopropanol	Fisher Scientific	Cat# A4644
Sodium acetate	Millipore Sigma	Cat# S8750
Glycerol	Genesee	Cat# 18-205
Ethanol	Decon Labs	Cat# V1016
Streptomycin	Fisher Bioreagents	Cat# BP910-50
Experimental Models: Organisms/Strains		
Mouse: CD1 IGS	Charles River Laboratories	N/A
Mouse: C57/BL6	UCR gnotobiotic facility	N/A
Other		
Coy vinyl anaerobic chamber system	Coy Lab Products	N/A
Vial, large opening crimp-top 2.0 mL	Chemglass Lifesciences	Cat# 50230754
Crimp-seal, 11 mm/red rubber septa	Chemglass Lifesciences	Cat# 50-153-2572
125 mL flask	Fisher Scientific	Cat# S63270
Commode Specimen Collection System	Fisher Scientific	Cat# 02-544-208
Intramedic PE Tubing .111NID 1 × 100 ft	BD	Cat# 1417012P
PrecisionGlide single-use needles 30 g	BD	Cat# 1482113A
1 mL syringe	BD	Cat# BD-309628
500 m: wide-mouth polypropylene jar with lid	Fisher Scientific	Cat# 02-891E
50 mL polypropylene beaker	Fisher Scientific	Cat# 1205-0050
General-purpose pinning forceps (4.5 inch)	Fisher Scientific	Cat# 10270
Dissecting scissors	Fisher Scientific	Cat# 08-951-10
2 mL sterile microcentrifuge tubes with screw caps	Fisher Scientific	Cat# 2681374

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Tissue homogenizer	Biospec	Cat# 985370
0.1 mm zirconia/silica beads	Biospec	Cat# NC0362415
Incubator (gravity convection)	Fisher Scientific	Cat# 51028112
Sterile cell strainer 70 µm	Fisher Scientific	Cat# 22-363-548
0.22 µm 500 mL Steritop filter	Millipore	Cat# SCGVT05RE
Fisherbrand Commode Specimen Collection System	Fischer Scientific	Cat# 02544208
Oligonucleotides		
Forward Primer: 5′-CTCCTACGGGAGGCAGCAG-3′	Alavi et al., 2020	N/A
Reverse Primer: 5′-TTACCGCGGCTGCTGGCAC-3′	Alavi et al., 2020	N/A

MATERIALS AND EQUIPMENT

Reagent	Concentration	Amount (for 1 L)
LYH-BHI media		
BHI broth (granulated)	37 g/L	37 g
Yeast extract	5 g/L	5 g
Hemin	5 mg/L	5 mg
Cellobiose	1 g/L	1 g
Maltose	1 g/L	1 g
Cysteine	0.5 g/L	0.5 g
ddH_2O	–	to 1 L

Reagent	Concentration	Amount (for 1 L)
LB media		
NaCl	5 g/L	5 g
Tryptone	10 g/L	10 g
Yeast Extract	5 g/L	5 g
ddH_2O	–	to 1 L
Bacto-agar (for plates)	15 g/L	15 g

Note: LYH-BHI media can be stored under anaerobic conditions at room temperature (20°C–22°C) for up to 3 months in opaque or foil-wrapped containers. LB can be stored at room temperature (20°C–22°C) for up to 6 months.

STEP-BY-STEP METHOD DETAILS

Preparation of Suckling Mice for Gavage

Timing: 1 h on day 0

⚠ CRITICAL: Specific-pathogen free (SPF) mice need to be pre-treated with oral antibiotics for 24 h prior to introduction of bacteria. Gavage procedures for antibiotics and bacteria cultures or human fecal specimens are identical.

Note: All 30°C steps are in incubator with open water pan to maintain humidity.

1. Mice should be 4 days old at time of antibiotic administration. CD-1 suckling animals were purchased from Charles River Laboratories. Germ-free suckling animals were obtained from the UCR gnotobiotic animal facility. Similar results have been obtained with C57/BL6 background suckling animals, and specific mouse backgrounds may be more appropriate depending on the pathogen of interest.
2. Fast infants (separate them from the lactating dam) for 1.5 h before gavage. Place pups in 50 mL plastic beaker lined with paper-towel or Kimwipe in 30°C incubator with open water pan to maintain humidity.
3. During fasting period, prepare materials needed for gavage including isoflurane, 30-gauge plastic tubing, glycerol, 30G × 1 needles, and 1 mL syringe.
4. Target antibiotic dosing is 1 mg/g body weight streptomycin. Based on the average weight of 4 days old suckling mice, concentration of streptomycin in 50 μL gavage would be 75 mg/mL in ddH_2O. Gavage procedure for streptomycin is identical to that described in steps 6–12.

Preparation of Bacterial Gavage

Timing: 2 h on each day 1 and day 2

5. Place the 50 mL beaker containing fasted animals in 500 mL plastic jar (wide-mouth polypropylene jar with lid) lined with tissue paper or Kimwipe (Figure 1A).
6. Take a representative animal, place the plastic tubing to be used for gavage parallel to their body, and mark on the tube using permanent marker the distance from the tubing end represented by the snout to the stomach measurement (Figure 1B). During gavage, tubing will need to be inserted until this mark reaches the snout of the animal to ensure intra-gastric inoculation (Figure 2).
7. Absorb 150 μL of isoflurane into tissue paper in jar.

⚠ CRITICAL: This prevents direct exposure of skin to isoflurane, which can cause pain/discomfort.

⚠ CRITICAL: Work inside chemical fume hood when working with isoflurane.

8. Place suckling mouse to be inoculated in small interior container (Figure 1C), re-place the lid on the outer container, and wait 30 s (Figure 1D).

Note: For anaesthetization of 2 mice, use ~300 μL isoflurane.

⚠ CRITICAL: Be careful not to tighten the lid of the outer container.

9. Take the bacterial mixtures (either complex or cultured microbiomes with *V. cholerae*, see steps 8 or 15, and step 9 in Before you Begin) that have already been prepared by syringe from the vial. Place the tubing on the needle using forceps sterilized with 70% vol/vol ethanol (see Methods Video S1), and dip the marked area of the tubing in sterile 50% glycerol to lubricate tubing.

10. Carefully insert tubing into the mouth of the suckling mouse, aligning the trajectory of the tubing to match the esophagus (Figure 2).
11. Once the mark on tubing reaches the snout, inject 50 μL of the inoculum into stomach. For example of complete gavage procedure, see Methods Video S2.

12. After each gavage, place mouse back in 30°C incubator.
13. After 15 min to disperse any residual isoflurane, place infants with dam.

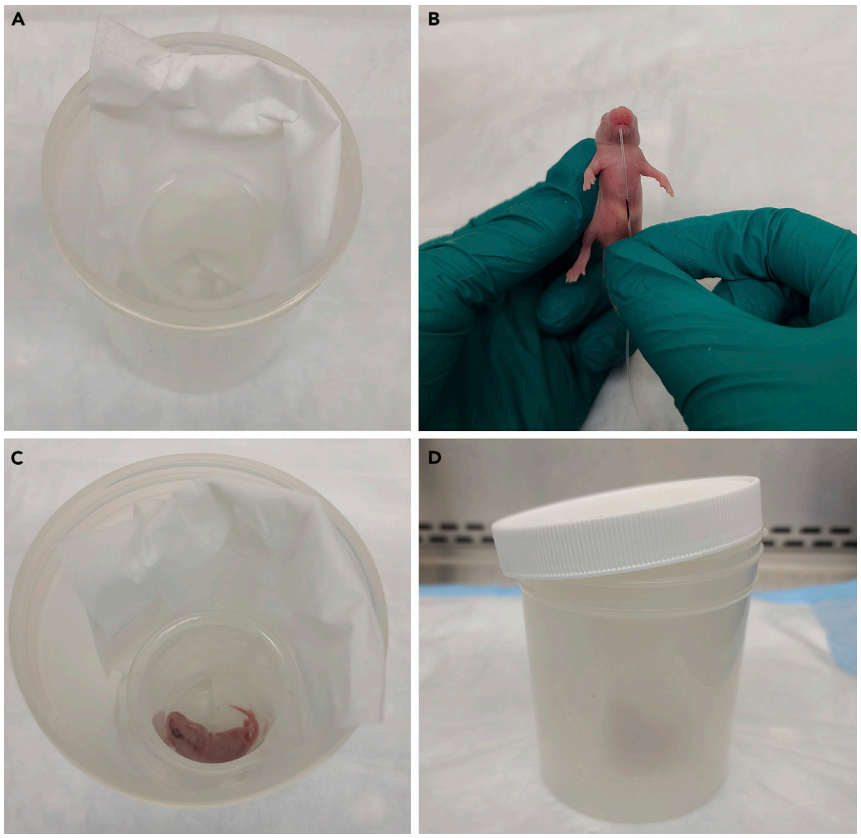

Figure 1. Preparing the Container Setup and Mice for Gavage

(A) A large container is lined with paper to absorb isoflurane.

(B) Measure and mark the gavage tube length needed to reach the stomach for intra-gastric gavage.

(C) Place 1–2 pups in a small container and place inside a large container to isolate from isoflurane.

(D) After absorbing the tissue in the outer container with isoflurane, place lid loosely and wait 30 s to anesthetize pups.

14. The next day, after preparing the cultures for gavage, fast the suckling mice in 30°C for 1.5 h.
15. Repeat the gavage with bacteria cultures/human fecal samples.
16. Place inoculated mice separated from dam in 30°C incubator for 15–16 h.

Mouse Dissection and Intestinal Homogenization

Timing: 4–5 h

17. Anesthetize the mice using the two-container method as described above for gavage, but add 1 mL isoflurane and close the outer lid tightly.
18. After 4–5 min, remove mice from container. Perform cervical dislocation to euthanize, and prepare for dissection.

⚠ CRITICAL: Disinfect all the forceps and scissors by ethanol (let them dry by air).

19. Spray 70% vol/vol ethanol on the surface of the body and allow it to air dry.
20. Make initial incision in the abdominal wall with dissection scissors (Figure 3). Continue excision laterally, taking care not to cut the intestine. Gently cut fascia binding the intestines so that they can be removed intact.
21. Once the intestines are moved out of the body cavity, cut at the proximal end where duodenum joins the stomach and at the distal end of the ileum.

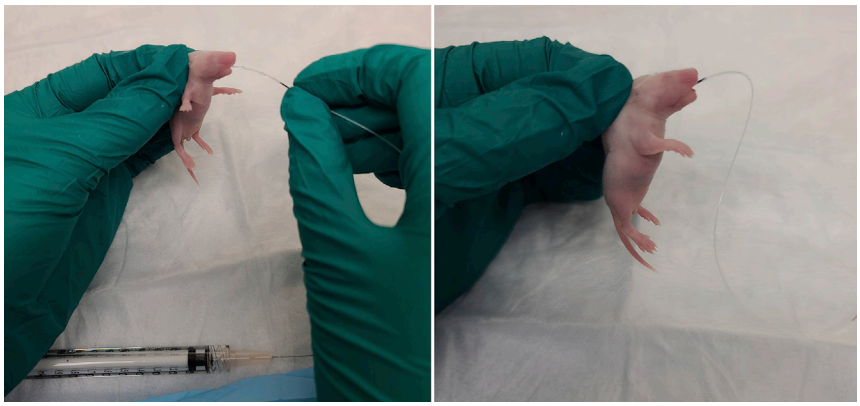

Figure 2. Gavage of Pups

22. Place the small intestine on the clean dissection tray. Divide the small intestine to three equal sections by length (Figure 4). For example of complete dissection procedure, see Methods Video S3.

23. Place each section in 5 mL sterile PBS.
24. Use tissue homogenizer to fully break the tissue.
    a. Initially pulse the homogenizer in sterile water for 30 s.
    b. Move to 100% vol/vol ethanol, and repeat.
    c. Pulse in third container of sterile water, and then homogenize tissue at full power for 60 s.

    Note: Repeat these steps (a, b, and c) after each homogenization.

25. To extract DNA from the homogenate sample.

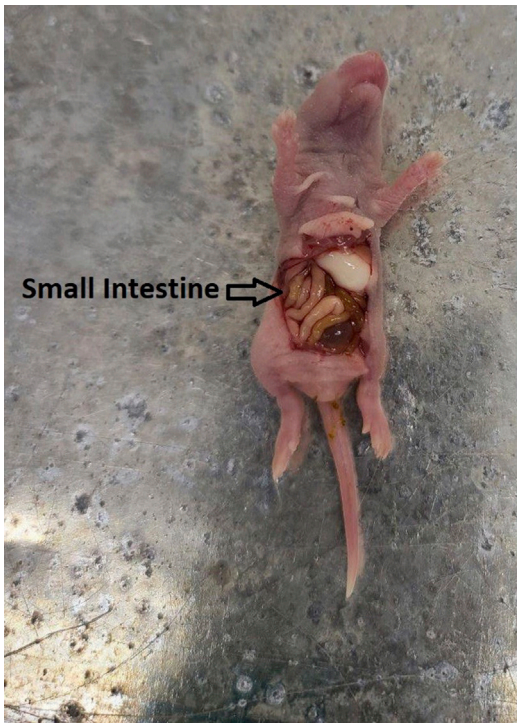

Figure 3. Mouse Dissection

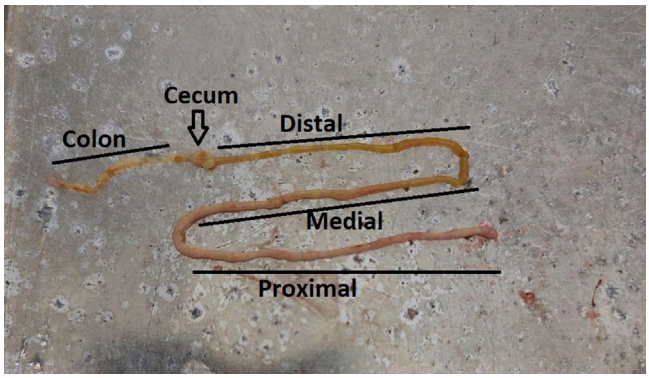

Figure 4. The Small Intestine Is Spread Out to Three Equal Sections Based on Length

a. Transfer 500 μL of homogenate to 2 mL screw-cap tube with ~500 μL 0.1 mm zirconia/silica beads (autoclaved).
b. Add 210 μL 20% wt/vol SDS.
c. Add 500 μL phenol:chloroform:isoamyl alcohol (25:24:1).
d. Bead beat on high speed for 2 min to disrupt tissue and lyse cells.
e. Centrifuge 16,000 × *g* at 4°C for 15 min.
f. Remove aqueous phase into new tube.
g. Add 500 μL phenol:chloroform:isoamyl alcohol, mix by inversion for 15 s.
h. Centrifuge 16,000 × *g* at 4°C for 15 min.
i. Remove aqueous phase into new tube.
j. Add 600 μL of −20°C isopropanol, 60 μL (1/10 volume) 3 M sodium acetate and mix by inversion.
k. Place in −20°C for 16 h or −80°C for 1 h.
l. Centrifuge at 16,000 × *g* for 30 min at 4°C.
m. Decant supernatant.
n. Add 500 μL 100% ethanol.
o. Decant supernatant.
p. Air dry at 37°C for 8–10 min.
q. Suspend pellet in 50 μL water by pipetting.

26. For CFU enumeration of *V. cholerae* in homogenate:
a. Add 10 μL of intestinal homogenate to 90 μL sterile PBS.
b. Serially dilute 10-fold until 10^{-4} dilution.
c. Plate 50 μL of the 10^{-3} and 10^{-4} dilution on the LB agar containing 200 mg/mL streptomycin.
d. Incubate plates at 30°C 16–18 h and count colonies to determine colonization level of *V. cholerae* (see Figure 5).

EXPECTED OUTCOMES

In this antibiotic-cleared mice model, the mice microbiome is ablated, allowing for the establishment of added human-associated bacteria in the mouse gut. The level of depletion can be quantified by extraction of DNA from intestinal homogenates followed by quantitative real-time PCR for microbial 16S genes using universal primers (Alavi et al., 2020). For a gavage of 1×10^4–1×10^5 CFU of *V. cholerae*, a colonization level of 10^6–10^8 CFU / small intestine is expected after overnight infection depending on the defined community used. The level of colonization for mice also receiving complex human fecal specimens range from 1.5×10^6–4×10^7 CFU.

LIMITATIONS

The method described here is only suitable for short term colonization experiments, as suckling mice cannot survive without long-term access to lactating dam. Replacing colonized pups with dam

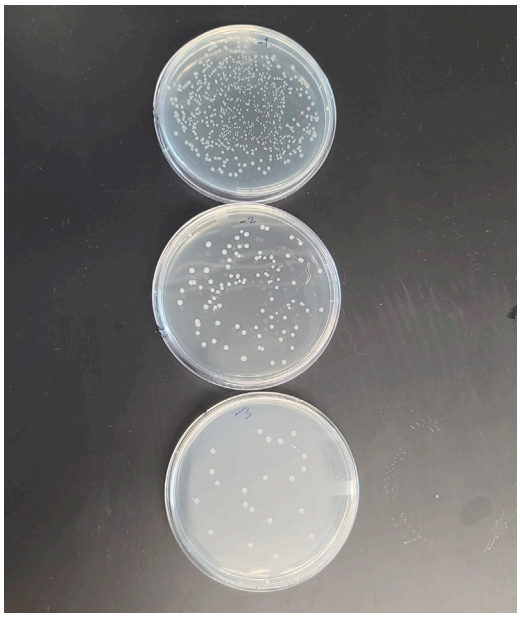

Figure 5. CFU Enumeration of *V. cholerae* by Serial Dilution and Plating

Top, 10^{-1} dilution; middle, 10^{-2} dilution; bottom, 10^{-3} dilution.

introduces differences in available nutrients and thus potentially different microbiome configurations, as diet is a potent driver of microbiome structure (Charbonneau et al., 2016). Non-dietary milk components are also able to directly interact with *V. cholerae* during infection in mice, potentially confounding microbiome-dependent results (Hsiao et al., 2006). Long-term colonization also risks cannibalization of sick pups before microbial data can be obtained. Previous studies involving continuous colonization with pups also have required substantially larger and more frequent administrations of *V. cholerae* to maintain colonization (Mao et al., 2018).

At the indicated doses of *V. cholerae*, the majority of microbes recovered after overnight infection are pathogens. For examination of microbiome-specific transcriptional or metabolomic activity during infection *in vivo*, the dosing of *V. cholerae* will need to be reduced. We also recommend the inclusion of uninfected control groups to examine *in vivo* commensal activity and viability. Intestinal samples from such animals can be used for 16S rRNA amplicon sequencing to determine the similarity of the transplanted community to the original fecal specimen.

The microbiomes endemic to individual animal facilities and backgrounds may differ, along with the presence of potentially streptomycin-resistant commensal organisms. Different antibiotic regimens may be required to ablate the murine microbiome prior to transplantation of human fecal specimens.

For the preparation of defined communities containing microbes requiring specific growth conditions, LYH-BHI may not be appropriate.

TROUBLESHOOTING

Problem 1

High levels of variability in colonization per mouse or failed colonization (step 11, Step-by-Step).

Potential Solution

Pups can regurgitate the gavage. To determine if the gavage is retained, add ~1 μL of food dye (filter-sterilized) to the gavage preparation. The inoculum will then be just visible in the stomach of pups under the skin.

Problem 2

Cannibalization of pups by dam after colonization (step 16, Step-by-Step).

Potential Solution

After gavage, make certain to disperse residual isoflurane before putting pups back with the dam. This can be accomplished by maintaining them for at least 15–20 min in incubator after gavage to make sure.

Problem 3

Mouse injury can happen during the gavage. Mouse deaths can happen from improper gavage (step 11, Step-by-Step).

Potential Solution

Suckling mice should be checked frequently in the hour after gavage to ensure that they are moving. Attrition due to gavage can lead to the loss of some animals, which should be accounted for in the number of animals available at the start of the experiment.

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Ansel Hsiao (ansel.hsiao@ucr.edu).

Materials Availability

Source of commercial mouse lines are indicated in Key Resources Table. Unique microbial strains and samples are available from the Lead Contact with a completed Materials Transfer Agreement.

Data and Code Availability

This study did not generate or analyze datasets/code.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.xpro.2020.100200.

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AUTHOR CONTRIBUTIONS

All authors designed and analyzed experiments. S.A. performed experiments. S.A. and A.H. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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