Video Article

Detection of Enterohemorrhagic *Escherichia Coli* Colonization in Murine Host by Non-invasive *In Vivo* Bioluminescence System

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Abstract

Enterohemorrhagic *E. coli* (EHEC) O157:H7, which is a foodborne pathogen that causes diarrhea, hemorrhagic colitis (HS), and hemolytic uremic syndrome (HUS), colonize to the intestinal tract of humans. To study the detailed mechanism of EHEC colonization *in vivo,* it is essential to have animal models to monitor and quantify EHEC colonization. We demonstrate here a mouse-EHEC colonization model by transforming the bioluminescent expressing plasmid to EHEC to monitor and quantify EHEC colonization in living hosts. Animals inoculated with bioluminescence-labeled EHEC show intense bioluminescent signals in mice by detection with a non-invasive *in vivo* imaging system. After 1 and 2 days post infection, bioluminescent signals could still be detected in infected animals, which suggests that EHEC colonize in hosts for at least 2 days. We also demonstrate that these bioluminescent EHEC locate to mouse intestine, specifically in the cecum and colon, from *ex vivo* images. This mouse-EHEC colonization model may serve as a tool to advance the current knowledge of the EHEC colonization mechanism.

Video Link

The video component of this article can be found at https://www.jove.com/video/56169/

Introduction

EHEC O157:H7 is a pathogen that causes diarrhea[1], HS[2], HUS[3], and even acute renal failure[4] through contaminated water or food. EHEC is a pathogenic enterobacterium and colonizes to the gastrointestinal tract of humans[1]. When EHEC first adhere to host intestinal epithelium, they inject the colonization factors into host cells through the type III secretion system (T3SS) that functions as a molecular syringe inducing an attaching and effacing (A/E) lesion subsequently to enforce adhesion (colonization)[5]. These genes involved in A/E lesion formation are encoded by the locus of enterocyte effacement (LEE) pathogenicity island[5].

Bioluminescence is a light-producing chemical reaction, in which luciferase catalyzes its substrate luciferin to generate visible light[6]. This enzymatic process often requires the presence of oxygen or adenosine triphosphate (ATP)[6]. Bioluminescence imaging (BLI) allows researchers the visualization and quantization of host-pathogen interactions in live animals[7]. BLI can characterize the bacterial infection cycle in live animals by following the bioluminescent bacteria as they migrate to and invade different tissues[7]; this reveals a dynamic progression of infection. Moreover, the bacterial load in animals is related to the bioluminescent signal[8]; thus, it is a convenient indicator to estimate the pathological conditions of experimental animals in a simple and direct way.

The plasmid used here contained the luciferase operon, *luxCDABE*, which is from the bacterium *Photorhabdus luminescens* that encodes its own luciferase substrate[7,9]. By transforming this luciferase-expressing plasmid into bacteria, the colonization and infection processes can be monitored by observing these bioluminescent bacteria in live animals. Overall, BLI and bioluminescence-labeled bacteria allow researchers to monitor the bacterial numbers and location, bacterial viability with antibiotics/therapy treatment, and bacterial gene expression in infection/colonization[6,7]. Numerous pathogenic bacteria have been reported that express the *luxCDABE* operon to examine their infection cycle and/or gene expression in infection. These bacteria, including uropathogenic *E. coli*[10], EHEC[8,11,12,13], enteropathogenic *E. coli* (EPEC)[8], *Citrobacter rodentium*[14,15], *Salmonella typhimurium*[16], *Listeria monocytogenes*[17], *Yersinia enterocolitica*[18,19], and *Vibrio cholerae*[20], have been documented.

Several experimental models have been developed to facilitate the study of EHEC colonization *in vitro* and *in vivo*[21,22,23]. However, there is a lack of suitable animal models to study the EHEC colonization *in vivo*, and thus a resulting paucity of details. To facilitate the study of the EHEC colonization mechanism *in vivo*, it is valuable to build animal models to observe and quantify EHEC colonization in live animals in a non-invasive method.

This manuscript describes a mouse-EHEC colonization model that uses a bioluminescent expressing system to monitor EHEC colonization over time in living hosts. Mice are intragastrically inoculated with bioluminescence-labeled EHEC and the bioluminescent signal is detected in mice with a non-invasive *in vivo* imaging system[13]. Mice infected with bioluminescence-labeled EHEC showed significant bioluminescent signals in

their intestine after 2 days post infection, which suggested that those bacteria colonized in the host intestine after 2 days post infection. *Ex vivo* image data showed that this colonization is specifically in the cecum and colon of mice. By using this mouse-EHEC model, the bioluminescent EHEC colonization can be detected in the living host by an *in vivo* imaging system, to study the detailed mechanisms of enteric bacteria colonization, which may promote further understanding in EHEC-induced physiological and pathological changes.

Protocol

Caution: EHEC O157:H7 is a biosafety level 2 (BSL-2) pathogen according to the Centers for Disease Control and Prevention (CDC) biosafety instruction (https://www.cdc.gov/). Therefore, all experimental procedures involving EHEC must be performed in a BSL-2 facility. Wear lab coats and gloves while performing the experiment. Work in a certified biosafety cabinet (BSC). Disinfect the experimental bench before and after the experimental procedure with 70% ethanol. All instruments or equipment that contact (or potentially contact) EHEC should be disinfected with 70% ethanol or bleach. Contaminated (or potentially contaminated) wastes should be sealed and autoclaved carefully. Wear a mask, eye protection, double gloves or a jumpsuit, if necessary. The 6-week-old C57BL/6 female mice were purchased and maintained at the Laboratory Animal Center of National Cheng Kung University (NCKU). The animal experiments were approved by the Institutional Animal Care and Use Committee of NCKU (Approval number 104-039).

1. Bioluciferase-labeled EHEC Bacteria Generation

1. Mix 50 ng deoxyribonucleic acid (DNA), 1 µL each of 10 µM forward and reverse primers, 2 µL 2.5 mM deoxynucleotides (dNTPs), 2 µL 10x buffer, 0.2 µL DNA polymerase, and sterile double-distilled water (ddH$_2$O) to a final volume of 20 µL to amplify the anti-kanamycin cassette, *nptII* gene[24]. The DNA template is from pBSL180[24], which is purchased from the National BioResource Project (NBRP). The PCR conditions are listed in **Table 1** and the primer sequence is available in **Table 2**.
2. Ligate the PCR products to a commercial cloning vector following the kit protocol (see **Table of Materials**).
3. Excise the *nptII* gene fragment from the cloning vector by *NsiI* and *SmaI* and clone into pBBR1MCS4, which is digested by the *NsiI* and *ScaI* from pAKlux2[9] to create the kanamycin resistant plasmid, pWF278[13].
4. Excise the *luxCDABE* operon, from the luciferase expressing plasmid[9], pAKlux2, by *SpeI-HF* and *ScaI* and clone to *SpeI-HF* and *SmaI* digested pWF278 to generate kanamycin resistant and luciferase expressing plasmid, pWF279[13].
    NOTE: For the plasmid extractions and the excised fragment purifications, use the commercial plasmid extraction and the gel extraction kit, respectively. For enzyme digestion, mix 100 ng DNA, 1 µL 10x buffer, 1 µL of each selected restriction enzyme, and sterile ddH$_2$O to a total volume of 10 µL, and incubate at 37 °C for 2.5 - 3 h.
5. Transform the pWF279 plasmid into *E. coli* O157:H7 EDL933 competent cells by electroporation with 2,500 V for 4 ms.
6. Incubate the transformed bacteria cells in 1 mL Luria-Bertani (LB) medium at 37 °C for 1 h.
7. Plate the bacteria on an LB agar plate supplemented with 50 µg/mL of kanamycin at 37 °C for 16 - 18 h.
8. Check the bioluminescent signal of the plate by an *in vivo* imaging machine the next day. Pick a single colony from the plate and culture it in 3 mL LB supplemented with 50 µg/mL kanamycin at 37 °C for 16 - 18 h.
9. Prepare bacterial stock medium by diluting 100% glycerol in sterile ddH$_2$O to make the 30% glycerol solution.
10. Freeze the kanamycin resistant *E. coli* O157:H7 EDL933 bacteria harboring the luciferase plasmid as a bacterial stock in a screw cap cryovial as a 1:1 ratio of bacteria culture and 30% glycerol solution at -80 °C. The final concentration of glycerol is 15%.

2. Bioluminescent EHEC Bacteria Preparation for Oral Inoculation

NOTE: The timeline flowchart of the experimental procedures for EHEC preparation and mouse oral gavage is presented in **Figure 1** to aid in experimental preparation.

1. Streak *E. coli* O157:H7 EDL933 bacteria harboring luciferase plasmid onto an LB agar plate with 50 µg/mL kanamycin from -80 °C stock. Grow the bacteria for 16 - 18 h at 37 °C.
2. Pick a single colony from the overnight plate and culture in 3 mL LB medium with 50 µg/mL kanamycin for 16 - 18 h in a 37 °C incubator at 220 rpm.
3. Subculture the bacteria (1:100 dilution) into kanamycin (50 µg/mL) containing LB broth for 2.5 - 3 h in a 37 °C incubator at 200 rpm. (For instance, add 2 mL overnight cultured bacteria to 200 mL LB medium with kanamycin (50 µg/mL) supplemented).
4. Incubate the bacteria for 2.5 - 3 h and measure the optical density value at 600 nm (OD$_{600}$) until the value is between 0.9 to 1. The bacterial cell number is at a density of about 10^8 colony forming units (CFU)/mL.
5. Centrifuge the re-cultured bacteria at 8,000 x g for 30 min, 4 °C.
6. Discard the supernatant without agitating the pellet of bacteria and wash the pellet with 100 mL 0.9% sterile normal saline by gentle agitation.
7. Repeat steps 2.5 and 2.6 once.
8. After the wash, centrifuge the bacterial culture at 8,000 x g for 30 min, 4 °C and discard the supernatant gently.
9. Condensate the pellet at 100-fold with 0.9% sterile normal saline. For instance, if 200 mL bacteria are centrifuged, add 2 mL normal saline to suspend the pellet.
10. Confirm the bacteria CFU (it should be approximately 10^9 CFU/100 µL after condensation in normal saline) by plating the concentrated bacteria through a 10-fold serial dilution[25].

3. Mouse Oral Gavage of EHEC

1. Treat 6-week-old female C57BL/6 mice with streptomycin water (5 g/L) for 24 h.
2. After 24 h, switch to regular drinking water for another 24 h before gavage. After treating with regular water for 24 h, the mice are ready for oral gavage of EHEC.

3. Fill the syringe with 100 µL EHEC bacteria by pulling back on the plunger. Ensure no air bubbles are in the syringe. Remove the bubbles by snapping the syringe with fingers.
4. Lift the animal gently and place it on the top of cage with care.
5. Grasp the mouse by the tail carefully, and the animal will grip the top of cage and attempt to move away.
6. Gently restrain the mouse by grasping the loose skin of the neck and back of the animal with thumb and forefinger to prevent the head of the mouse from moving.
7. Hold the mouse in a vertical position to insert the gavage needle and ensure the head of mouse is immobilized and vertical.
8. Insert the gavage needle into the mouse mouth following the roof of the mouth and move down into esophagus and toward the stomach.
9. When the inserted needle is half or two-thirds length in the mouse, inject the 100 µL EHEC bacteria, which contain approximately 10^9 CFU cells.

4. Visualization

1. After the oral gavage, detect the bioluminescent signals on 1 and 2 days.
2. Before examining the bioluminescent signals of animals, anesthetize them by putting them into a chamber of 2.5% isoflurane with 1.5 L/min oxygen.
3. Wait for 2 - 5 min until all mice become unconscious and stop moving. Animals are ready for *in vivo* detection of bioluminescence.
4. Detect and image bioluminescent signals of animals by an *in vivo* imaging system. Please see Section 5 "Data Acquisition" for software operation. During the imaging process, all animals are under a continued supply of 2.5% isoflurane with 1.5 L/min oxygen.
   1. Click file>live, and manually focus on the mouse.
   2. Select the exposure time and laser intensity.
   3. Click acquire>capture.
5. For *ex vivo* imaging, euthanize the mice by cervical dislocation and remove the entire intestine of infected mice. Place the intestines in a 9-cm Petri dish and image by the *in vivo* imaging system. The setting is the same as the *in vivo* imaging except the Field of View is set as A/B. Please see Section 5 "Data Acquisition" for details.
   NOTE: Method for cervical dislocation: Restrain the mice on the top of cage by grasping the tail with one hand so that the animals grip the cage. Place a marker pen or the thumb and first finger of the other hand against the back of the neck at the base of the skull. Quickly push forward with the hand or object while restraining the head and pull backward with the hand holding the tail.
   Check closely to confirm respiratory arrest, and no heartbeat.

5. Data Acquisition

NOTE: The software used for data acquisition is listed in the **Table of Materials**.

1. For image acquisition, open the Acquisition Control Panel of the software (**Figure 2**).
2. Select "Luminescent," "Photograph," and "Overlay."
3. Set Exposure Time as "Auto." Set Binning as "Medium."
4. Set ƒ/stop as 1 for luminescent and 8 for photograph. ƒ/stop controls the amount of light received by the CCD detector.
5. Set the Field of View based on the field of images that are of interest to acquire. Option "D" can fit five 6-week-old mice and image them all at a time. "C" can image three 6-week-old mice in one field.
6. Once the mice/samples are ready for imaging, click "Acquire" for image acquisition.
7. Open the image data that was acquired.
8. Open the Tool Palette panel (**Figure 3**).
9. Select ROI Tools. We recommend the Circle (the left-most one) to range the bioluminescent area on images (**Figure 4**).
10. Click "Measure ROIs" (pencil icon) to measure the surface bioluminescent intensity (**Figure 3**). The ROI Measurements panel and the quantification values appear (**Figure 5**).
11. Use the Configure Measurement on the left corner of the ROI Measurements panel to select the values/information needed (**Figure 6**), otherwise click "Export" to export this data table and save as .csv file (**Figure 5**).
12. Use the values of column "Total Flux (p/s)" as the bioluminescent intensity quantification in the .csv file.

Representative Results

We administered bioluminescence-labeled EHEC (~ 10^9 bacterial cells) to 6-week old female C57BL/6 mice by oral gavage. After oral inoculation of EHEC to mice within 1 h, the animals were examined for bioluminescent signal by the *in vivo* imaging system as shown in **Figure 7**. The results showed a strong bioluminescent signal in gavage mice with bioluminescence-labeled EHEC. We examined the signals on 2 days post infection. As shown in **Figure 8A**, the mice inoculated with bioluminescence-labeled wild-type EHEC EDL933 showed intense bioluminescent signals even after 2 days post infection, which suggested EHEC colonized in hosts by 2 days. We also intragastrically infected bioluminescence-labeled EDL933*ΔrfaD (ΔrfaD)* to mice (**Figure 8A**). This mutant, defected in lipopolysaccharide (LPS), has been shown to reduce colonization in the host in our previous study. As shown in **Figure 8A**, there is no bioluminescent signal detected in *ΔrfaD*-infected mice, which suggests that there are no or less bacteria cells colonized in the mice. Quantification of the fluorescent signal is shown in **Figure 8B**. Next, the location of these bioluminescence-labeled bacteria was determined. The infected mice were sacrificed humanely and their whole intestine removed. The intestines of mice 2 days post infection were positioned on 9 cm Petri dishes and imaged *ex vivo* (**Figure 9A**). The intestinal tissues of bioluminescence-labeled EDL933 infected mice revealed a significant increase in bioluminescent signals in the cecum and colon, which suggest that these bioluminescent EHEC colonized in the cecum and colon of infected mice for 2 days at least. In contrast, mice infected with bioluminescence-labeled *ΔrfaD* (**Figure 9A**)*,* revealed decreased bioluminescent signal in their intestinal tissue, which is consistent with the *in vivo* image (**Figure 8A**). Quantification of the fluorescent signal is shown in **Figure 9B**.
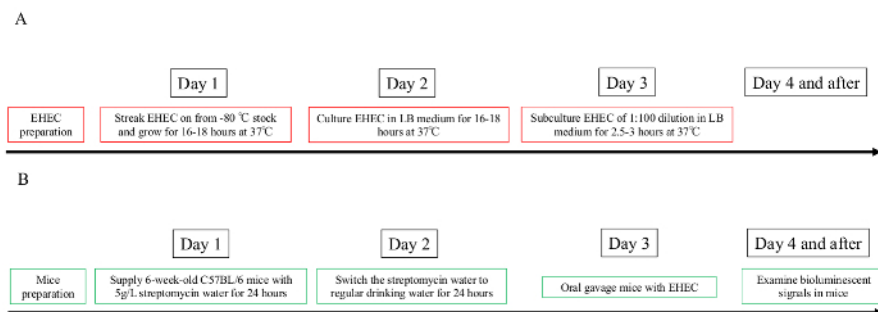
A

B

Figure 1: Timeline of the experimental preparation flow chart.
Overview of the timing needed to prepare bioluminescent EHEC bacteria and pretreat mice with streptomycin. (**A**) EHEC preparation. (**B**) Mice preparation. Please click here to view a larger version of this figure.
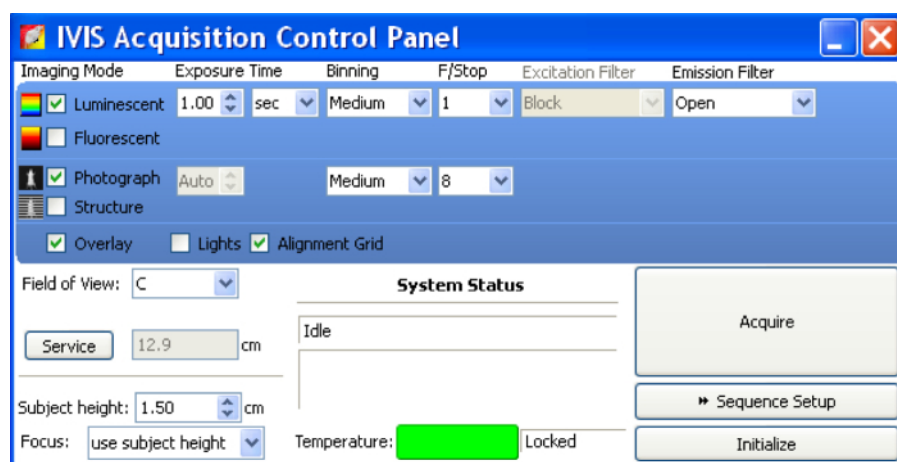

Figure 2: *In vivo* imaging system acquisition control panel.
Before imaging samples, open IVIS Acquisition Control Panel. Select "Luminescent," "Photograph," and "Overlay." Set Exposure Time as "Auto." Set Binning as "Medium." Set ƒ/stop as 1 for luminescent and 8 for photograph. ƒ/stop controls the amount of light received by the CCD detector. Once samples are ready for imaging, click "Acquire" to acquire images. Please click here to view a larger version of this figure.
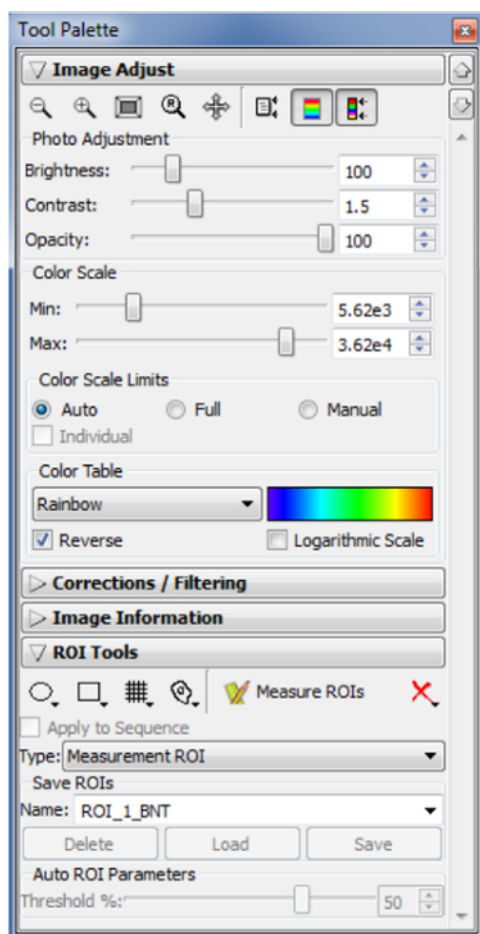
Figure 3: Tool Palette panel.
After image acquiring, use the Tool Palette panel for quantifying bioluminescent intensity. Open the Tool Palette panel and image the data. Choose one of the ROI Tools to range the bioluminescent signals on images. Please click here to view a larger version of this figure.
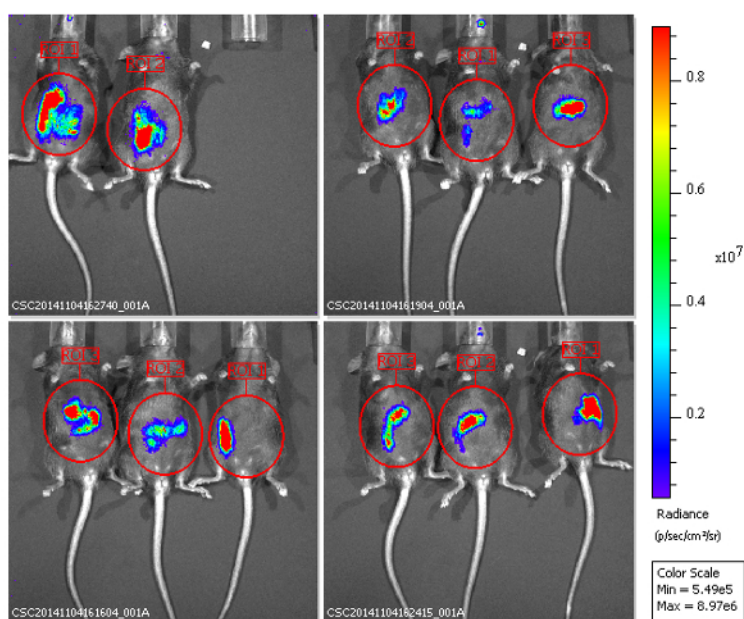

Figure 4: Bioluminescent signal from sample for quantification.
Bioluminescent signal area on images encircled by ROI Tools. All bioluminescent signals shown here are in the red circle. Please click here to view a larger version of this figure.
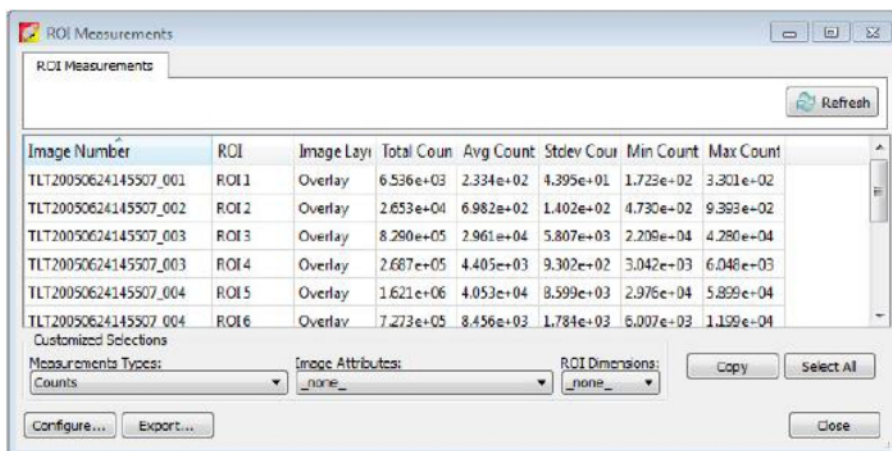
Figure 5: ROI measurements.
After circling bioluminescent signals and clicking "Measure ROIs" on the Tool Palette panel, values are presented as shown. The values of the column Total Flux (p/s) are used for the bioluminescent intensity quantification. Please click here to view a larger version of this figure.
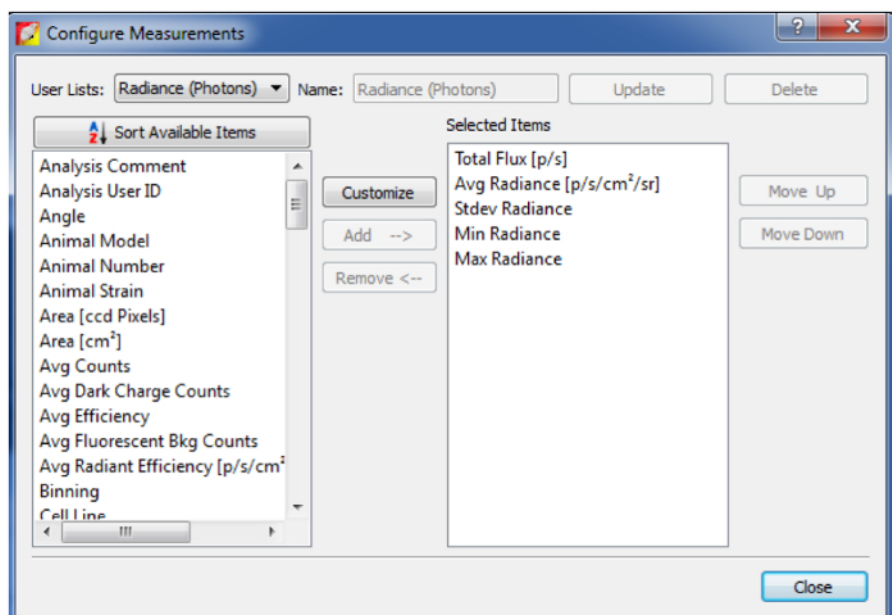

Figure 6: Add different quantification information.
By clicking on Configure Measurement on the left corner of the ROI Measurements panel, you can select other desired quantification values/information. Please click here to view a larger version of this figure.
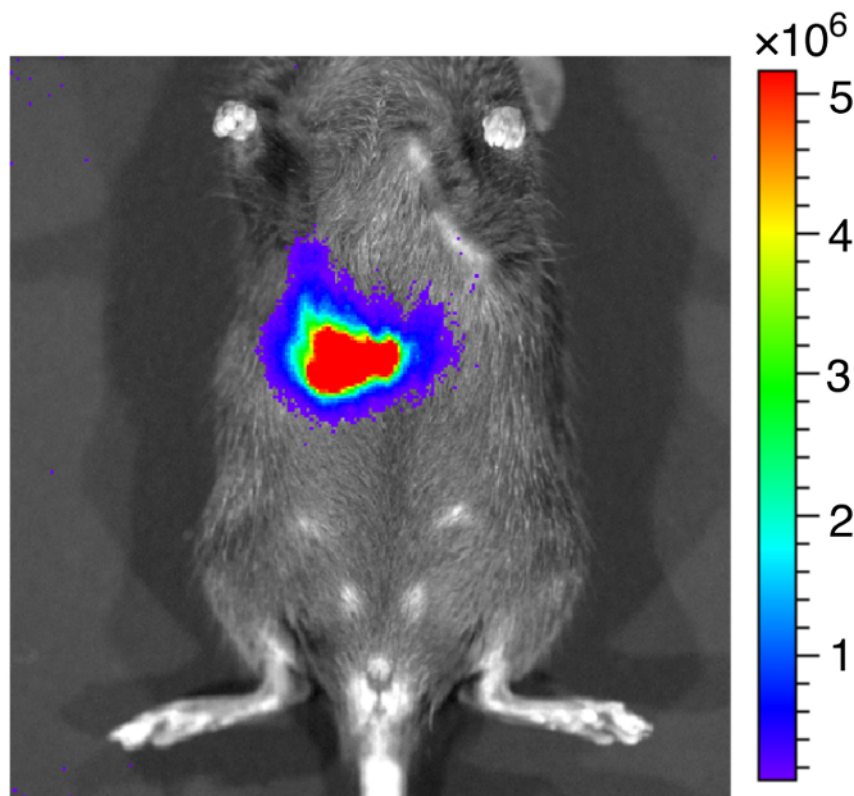
Figure 7: Representative image of mice after inoculated with bioluminescent EHEC.
Representative image of mice inoculated with bioluminescent EHEC by oral gavage within 1 h. The color scale represents the radiance (p/s/cm^2/sr). Please click here to view a larger version of this figure.
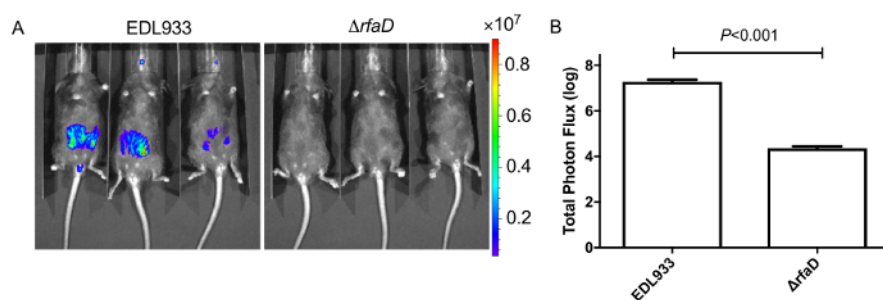

Figure 8: Images of mice inoculated with bioluminescence-labeled EHEC after 2 days.
(**A**) Represent image of mice inoculated with bioluminescent wild-type EHEC EDL933 and EDL933:$\Delta rfaD$ by oral gavage after 2 days post infection. (**B**) Quantification of bioluminescence intensity of mice infected with EHEC. Error bars indicate the standard deviations. Representative images are shown. All experiments were conducted independently three times with 2 - 3 animals each time, and error bars indicate the standard deviations. P-values denote the results of statistical analysis by t-test. The color scale represents the radiance (p/s/cm^2/sr). Please click here to view a larger version of this figure.
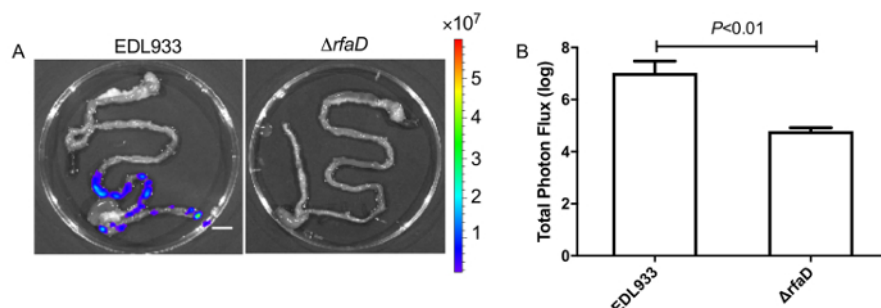
Figure 9: Images of intestinal tissues of infected mice with bioluminescence-labeled EHEC.
(**A**) 2 days after inoculation with bioluminescence-labeled EHEC, the mice were euthanized and whole intestinal tissues were removed and imaged *ex vivo*. Representative images are shown.(**B**) Quantification of bioluminescence intensity of intestinal tissues from mice infected with EHEC. All experiments were conducted independently three times with 2 - 3 animals each time, and error bars indicate the standard deviations. P-values denote the results of statistical analysis by t-test. The color scale represents the radiance (p/s/cm^2/sr). Scale bar represents 1 cm. Please click here to view a larger version of this figure.

Steps	Temperature	Time	Number of cycles
Initial denaturation	95 °C	10 min	1
Denaturation	95 °C	30 sec	35
Annealing	58.4 °C	30 sec	
Extension	72 °C	1.5 min	
Final extension	72 °C	10 min	1
Hold	4 °C	∞	1

Table 1: Polymerase chain reaction (PCR) conditions

Primers name	Sequence
nptII F	5'CCTATGCATAATAATTCCGCTAGCTTCACG3'
nptII R	5'GCTCCACCGATAATATTCCTGAGTCATACT3'

Table 2: Primer sequences used to amplify *nptII*

Discussion

It has been reported that EHEC transformed with luciferase plasmid has been utilized to examine its localization in hosts or gene expression *in vivo*[8,11,12]. The murine model demonstrated here has also been reported to detect the EHEC colonized timing and localization in murine host[8]. Nevertheless, we provide the detail protocol of how to administer EHEC inoculation to mice intragastrically and how to carefully prepare the bioluminescent bacteria for oral gavage. Notably, for the mouse oral gavage of EHEC (step 3.7), the position of the mouse head is critical when the gavage needle is inserted. If the position is not vertical, it will be difficult to pass the needle, and it could possibly injure the mouse. In step 3.8, when the gavage needle is inside the mouth of the mouse, the tongue will lay outside of the mouth slightly. If resistance is encountered when passing the gavage needle to the esophagus, stop moving the needle forward and withdraw it immediately. Alter the needle position to make sure that the needle is entering the esophagus. The needle could be entering the trachea when resistance occurs, which would lead to injecting bacteria in the lungs instead of the stomach.

Application of green fluorescent protein (GFP) as a biosensor is common in biological experiments. However, using GFP as a reporter to observe the pathogen infection/colonization in live animals by *in vivo* imaging is not recommended, because the absorbance by hemoglobin, proteins and water are high between 200-650 nm[26], which overlaps with GFP (excitation 480 nm, emission 510 nm)[27]. Therefore, using GFP signal as a reporter for *in vivo* imaging can be interrupted by hemoglobin, proteins, and water in animals[26]. The near-infrared (NIR) fluorescence is ideally suited for *in vivo* imaging because its absorbance window is around 650 - 900 nm[28], which is in the region of lowest absorption coefficient of hemoglobin (<650 nm) and water (>900 nm)[26,28]. Moreover, when tissue absorbs light, there is a chance to induce autofluorescence. When the wavelengths of excitation and emission range in the GFP window, it induces much more autofluorescence than NIR[29]. Use of NIR can improve the signal to background ratio compared to that of GFP by eliminating the autofluorescence background[29]. Bioluminescence does not require energy excitation to generate visible light. It depends on the reaction to catalyze substrate luciferin by its enzyme luciferase and generate light. Since bioluminescence does not require light directly on a sample, the background signal from a sample is very low. Therefore, use of bioluminescence as a reporter is more general and easier for *in vivo* imaging. In contrast, fluorescence requires light excitation to induce signal light. When tissues absorb light, there is a chance that the fluorescent light will be emitted and induce autofluorescence so that their signal-to-noise is higher compared to that of bioluminescence.

Considering EHEC is naturally less colonized in mice by oral infection[2,22], a natural mucosal pathogen of mice, called *Citrobacter rodentium*, has been utilized to study the colonization mechanism to murine host as a surrogate bacterium[22,30]. Both of EHEC and *C. rodentium* colonize the intestinal mucosa and induce the formation of A/E lesions in host[22,30]. They also contain the LEE pathogenicity island, which encodes a T3SS

and several effector proteins that induced A/E lesion[22,30]. Therefore, the use of luciferase expressing plasmid as a reporter in *C. rodentium* to detect the colonization pathology and study the colonization mechanism via an *in vivo* imaging system has also been reported[14,15]. Nevertheless, while *C. rodentium* infection of mice is a useful model to investigate the function of T3SS and the mechanism of A/E lesion, *C. rodentium* does not contain Shiga toxin (Stx)[30], which is a dominant virulence factor that causes kidney failure in EHEC, particularly serotype O157:H7[3]. Although a Stx-expressing *C. rodentium* strain has been constructed recently[31], which is more realistic to EHEC infection, it does not include other potential EHEC virulence factors that are crucial for colonization and/or infection. Furthermore, *C. rodentium* shares 67% of its genes with EHEC[32], which suggests that EHEC may use a virulence distinct from *C. rodentium* during colonization and/or infection.

The luciferase expressing plasmid used in here, pWF279[13], was modified from pAKlux2[9] whose backbone is pBBR1MCS4[33]. Although pBBR1MCS4 have been tested and replicated in various bacteria[33], it is crucial to ensure the origin of replication (ORI) of this plasmid is suitable for the bacterial host before using this plasmid-based luciferase system for the experiment and thus confirming that this luciferase expressing plasmid can replicate in the bacterial host. We use antibiotic stress to maintain the stability of plasmid in the bacteria. When bacteria enter animals in the absence of antibiotics, the bioluminescent signal of wild-type EHEC has been detected for at least 2 days. However, we didn't following infection for longer than 2 days because we had already seen a significant difference in luminescent intensity between EHEC WT and EHEC *rfaD* (that encodes a gene required for EHEC synthesizing intact LPS) mutant at 2 days. To maintain the plasmid stably in bacteria under the absence of antibiotics, a plasmid pCM17[10,34] can be used for this purpose. pCM17 encodes a two-plasmid partitioning system and a post-segregational killing mechanism to ensure the maintenance of the plasmid in bacteria in the absence of antibiotics[10,34]. The plasmid pCM17 containing the *luxCDABE* operon driven by the OmpC promoter can be detected by bioluminescent signal for at least 7 days[8]. An alternative method to obtain a continuous bioluminescent expression bacteria in the absence of antibiotics is to insert *luxABCDE* gene into the bacterial chromosome[35]. Francis *et al.* used transposon inserted the *luxABCDE* operon and antibiotics cassette randomly inserted into the chromosome of *Streptococcus pneumoniae* to obtain the bioluminescence stable strain[35].

In our previous study[13], we utilized this model system to examine EHEC colonization in a host and compare the difference of colonization ability between the EHEC wild type (WT) and mutant[13]. When mice were administered the bioluminescent EHEC *rfaD* mutant, the bioluminescent signals diminished dramatically compared to that of WT EHEC after 2 days post infection. It provides evidence that this murine model can analyze the mutation effect of EHEC colonization in the host. Furthermore, therapeutic treatments for reducing the colonization of EHEC is a considered, potential solution to EHEC infection since the use of antibiotics is contraindicated[5,36]. Therefore, it is worth testing whether this model system can be used to examine the efficacy of anti-colonization drugs/treatments against enterobacteria colonization in the host. We believe that by using this model system, it is possible to examine the timing and location of not merely EHEC, but also other enterobacteria colonization *in vivo*. By using this animal model, the process of EHEC colonization in mice can be monitored and the colonization burden in the host can be quantified to determine spatial and temporal colonization of EHEC in live animals. The visualization and quantification of enterobacteria colonization by using this model makes it a great tool to investigate and analyze the fine mechanisms of enterobacterial colonization, and thus compensate for the deficiency of colonization research and improve current knowledge.

Disclosures

The authors have nothing to disclose.

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