Methods in Molecular Biology 1734

Springer Protocols

Carlos Medina
Francisco Javier López-Baena *Editors*

Host-Pathogen Interactions

Methods and Protocols

Humana Press

Chapter 4

Usage of a Bioluminescence Reporter System to Image Promoter Activity During Host Infection

Gili Aviv and Ohad Gal-Mor

Abstract

Bioluminescence is the process of production and emission of light by a living organism, usually as the by-product of the oxidative enzyme, luciferase. Currently available technology allows for the exploitation of a bioluminescent reporter system to study bacterial gene regulation during rodent infection, in real time, over a large dynamic range. Here we show how this imaging system can be used to study virulence gene regulation during *Salmonella enterica* infection in the mouse model. To demonstrate this technique we show the ex vivo expression pattern of the gene *dksA,* encoding a conserved and pleotropic regulator, which plays a key role in *Salmonella* pathogenicity [1].

Key words Luciferase, Bioluminescence, Reporter gene, In vivo imaging, Transcriptional regulation, Host infection

1 Introduction

Salmonella enterica is a gram-negative pathogen that is able to infect and cause disease in a wide range of animal hosts including human. Hundreds of genes scattered along its genome are involved in the virulent lifestyle of *S. enterica* and its complex interactions with diverse hosts. These virulence genes are tightly regulated and their expression is orchestrated according to a multifarious net of cellular and environmental signals. Thus, studying virulence gene regulation is essential to understand *Salmonella* biology and its pathogenic nature. The production of bioluminescence and the technology enables to quantity its signal can be now utilize to follow after pathogens gene expression during the infection.

Production and emission of light by a living organism is called bioluminescence. Different marine and terrestrial bacteria including the *Vibrio*, *Photobacterium*, and *Xenorhabdus* genera harbor the *lux* genes encoding for the oxidative enzyme luciferase (*luxAB*) and the fatty acid reductase system (*luxCDE*) required for the synthesis of the fatty aldehyde, which is used as the substrate for the

Carlos Medina and Francisco Javier López-Baena (eds.), *Host-Pathogen Interactions: Methods and Protocols*, Methods in Molecular Biology, vol. 1734, https://doi.org/10.1007/978-1-4939-7604-1_4,

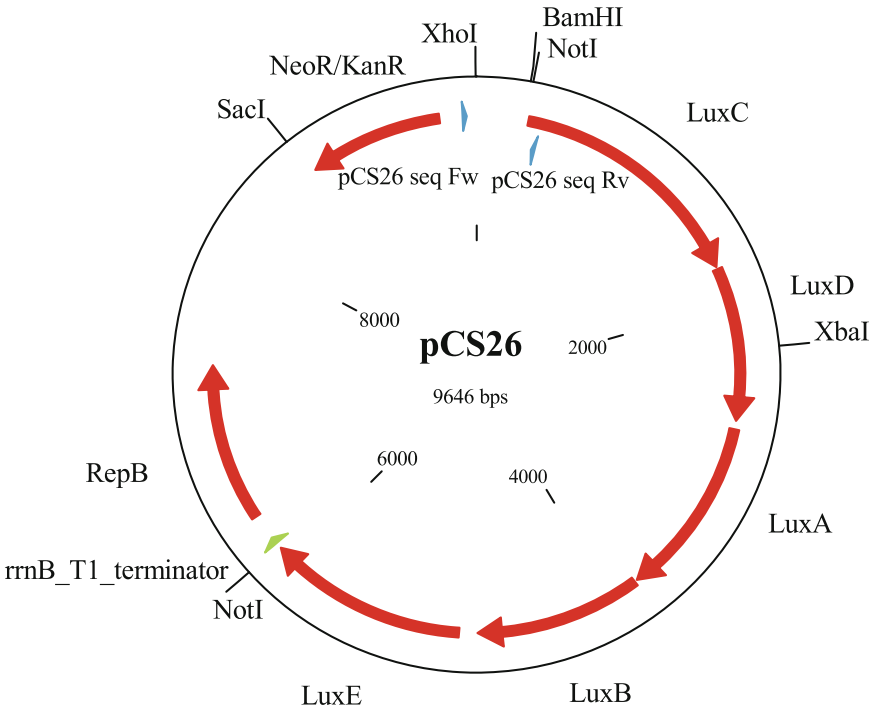

Fig. 1 pCS26 Map. The *lux* operon (*luxCDABE*) and the cloning sites upstream to the *lux* operon (XhoI, BamHI, and NotI) are indicated. Sequencing primers are shown as blue arrow heads. Construction of this vector was reported in reference [3]

luminescence chemical reaction [2]. A recently developed technology involves an ultra-sensitive CCD camera that can detect the light emission produced during bioluminescence and uses this signal for noninvasive imaging of small laboratory animals.

A self-sufficient bacterial luminescence-based reporter system has been developed by Bjarnason and colleagues [3]. This reporter plasmid (termed pCS26) contains a promoter cloning site upstream to the *luxCDABE* operon of the bacterium *Photorhabdus luminescens* [2] inserted into the vector pSC101 (Fig. 1). The advantage of this system is that it does not require any additional substrate to produce light when it is expressed from an active promoter. Thus, introducing a functional promoter of interest, (containing the upstream regulatory region and the RNA polymerase binding site of a target gene) results in light production when the promoter is turned on. This bioluminescent system generates light at a wavelength of 490 nm [4] and has a large dynamic range, enabling detection and quantification of a very weak to a very strong transcriptional activity. This approach was proved to be a very useful tool in studying promoter activity in vivo, during mouse infection of different bacterial pathogens [1, 5–7] (*see* **Note 1**).

To study the expression pattern of *dksA* we cloned its native regulatory region upstream to the *luxCDABE* operon and

introduced this construct (pCS26::p*dksA*) into wildtype *S.* Typhimurium. The empty vector was used as a negative control. C57BL/6 mice were orally infected with these *S.* Typhimurium reporter strains and 24 h postinfection they were sacrificed and imaged for bioluminescence along their gastrointestinal tract (Fig. 2) [1].

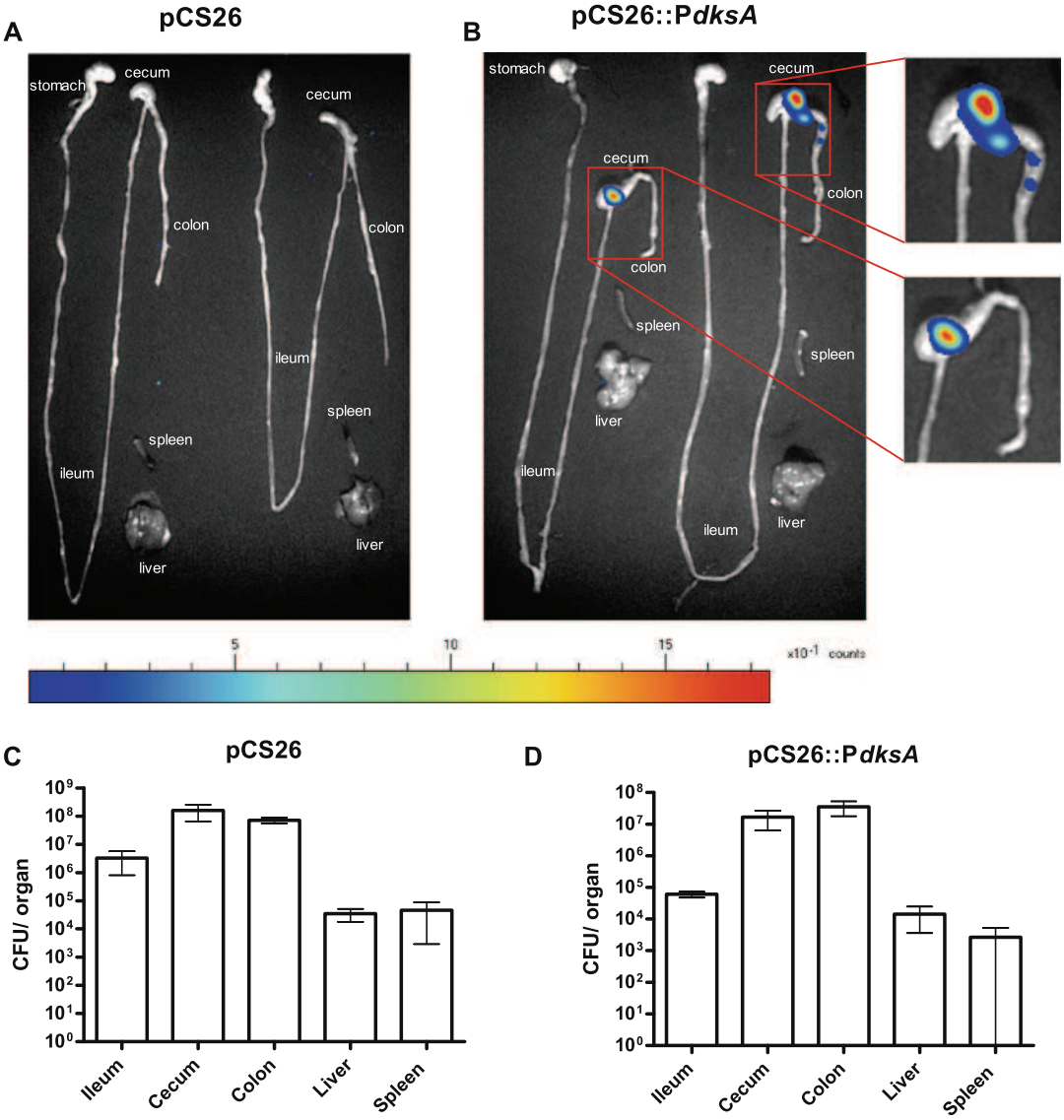

Fig. 2 *dksA* is expressed at the mid-cecum during intestinal colonization in vivo. Streptomycin-pretreated C57BL/6 mice were infected with 5–8 $\times$ 10^6 CFU of *S.* Typhimurium harboring pCS26 (**a**) or pCS26::p*dksA* (**b**) 24 h.p.i. the intestinal tract and systemic sites (liver and spleen) were removed and bioluminescence was imaged using a photon-counting system. Organs from two mice are shown from each infection. To determine the total numbers of colonizing *Salmonella* (CFU), organs were homogenized in saline, diluted and spread plated on XLD agar supplemented with kanamycin. Bars represent the mean bacterial loads and SEM in three mice infected with *S.* Typhimurium carrying pCS26 (**c**) or pCS26::p*dksA* (**d**). This figure is reproduced from Azriel et al. 2016 [1] with permission from the publisher (ASM Journals)

2 Materials

1. Luria–Bertani (LB) broth (Lennox) For 1 L: 10 g tryptone, 5 g yeast extract, 10 g NaCl (pH 7.0).
2. Bacterial genomic DNA purification kit.
3. Plasmid mini extraction kit.
4. Hot start high fidelity DNA polymerase.
5. Appropriate restriction enzymes.
6. DNA gel extraction kit.
7. T4 DNA ligase.
8. Oligonucleotides for sequencing:
   pCS26 seq Forward: 5′- CCGACGTCTAAGAAACCATTATTATC-3′.
   pCS26 seq Reverse: 5′- CACTAAATCATCACTTTCGGGAAAG-3′
9. 6–8 week old C57BL/6 female mice (*see* **Note 2**).
10. Stainless feeding needles (24G-1″ straight 1.25 mm ball).
11. Surgical scissors and tweezers.
12. Streptomycin and kanamycin.
13. HEPES buffer.
14. Saline (0.9% w/v of NaCl in dH_2O).
15. In vivo imaging instrument suitable for bioluminescence imaging [e.g., PhotonIMAGER (BIOSPACE LAB) or IVIS Spectrum In Vivo Imaging System (PerkinElmer)].
16. BeadBlaster 24 microtube homogenizer or any other microtube homogenizer machine.
17. Xylose lysine deoxycholate (XLD) agar plates.

3 Methods

3.1 Cloning the Target Promoter in the Bioluminescence Reporter System

1. Extract the appropriate bacterial genomic DNA that will be used as the template for the PCR by bacterial genomic DNA purification kit or any other standard method.
2. Isolate the reporter vector pCS26 [3] (Fig. 1) DNA using a plasmid mini extraction kit.
3. Conduct a PCR to amplify the regulatory region controlling the expression of the target gene (*see* **Note 3**). Make sure to include appropriate restriction sites (e.g., XhoI and BamHI) at the 5′- and 3′-ends of the amplified DNA.
4. Clean the resulted PCR product from an agarose gel using a gel extraction kit.

5. Cut the pCS26 and the PCR product with XhoI and BamHI.
6. Apply a DNA purification step to remove uncut DNA, enzymes and salts that were left from the restriction reaction (*see* **Note 4**).
7. Ligate the insert into the cut pCS26 using T4 DNA ligase at 16 °C overnight.
8. Transform the ligated plasmid into competent *Escherichia coli* DH5α cells.
9. Confirm the presence of the target insert by restriction analysis and DNA sequencing using the oligonucleotides "pCS26 seq Forward" and "pCS26 seq Reverse."
10. Isolate the reporter plasmid from the surrogate *E. coli* and introduce the plasmid by electroporation into an appropriate electrocompetent *Salmonella* cells.

3.2 Mice Infection and Imaging

1. Twenty-four hours prior to the infection administrate the mice with streptomycin (20 mg per mouse) by oral gavage in 100 μL HEPES buffer (100 mM, pH 8.0) (*see* **Note 5**).
2. Grow the *Salmonella* reporter strains in LB supplemented with kanamycin (50 μg/mL) at 37 °C with aeration for overnight.
3. Infect the mice orally with ~5–7 × 10^6 CFU of the reporter strains suspended in 0.2 mL of saline.
4. Twenty-four hours postinfection (or at later time points if needed) sacrifice the mice and remove their intact gastrointestinal tract as well as systemic organs (e.g., liver and spleen) and place them on a black nonreflective cardboard sheet inside the imaging machine in order to record the luminescence signal (Fig. 2).
5. After imaging, isolate the gastrointestinal tract organs (e.g., ileum, cecum and colon) and homogenize them in 700 μL of saline using a microtube homogenizer.
6. Prepare serial dilutions (0–10^{-6}) in saline and plate them on XLD agar plates supplemented with kanamycin (50 μg/mL) for CFU count.
7. Count the colonies and calculate the bacterial load per organ (*see* **Note 6**).

4 Notes

1. This assay can be used not only for in vivo detection of gene expression, but also for examining gene expression under different environmental conditions and growth phases in culture using a luminometer. In this case gene expression can be

expressed as the luminescence normalized to optical density at OD_{600}.

2. For each reporter strain use at least 4–5 mice as some variation in *Salmonella* colonization is not uncommon.
3. For positive control, clone the promoter of a constitutively expressed gene such as *rpoD* and for negative control use the empty (promoter-less) vector.
4. It is recommended to desalt the cut inserts by ethanol precipitation (2.5 volume of ice-cold ethanol absolute; 0.1 volume of 3 M sodium acetate pH 5.2; and 5 μg of yeast tRNA). The cloning vector needs to be purified by a gel extraction kit in order to remove the uncut vector molecules.
5. Pretreating the mice with streptomycin prior to infection diminishes the mouse microflora and allows better *Salmonella* colonization in the gut.
6. CFU count in organs is important to show that low or no luminescence, is not due to poor colonization, but instead reflects the expression pattern of the target promoter.

Acknowledgments

The research in Gal-Mor lab is supported by a grant number 1096.39.11/2010 from the German-Israeli Foundation for Scientific Research and Development (GIF); by a grant number 999/14 from the Israel Science Foundation (ISF) and by grant number 3-0000-12435 from Infect-ERA and the Chief Scientist's Bureau in the Israeli Ministry of Health.

References

1. Azriel S, Goren A, Rahav G, Gal-Mor O (2016) The stringent response regulator DksA is required for *Salmonella enterica* serovar Typhimurium growth in minimal medium, motility, biofilm formation, and intestinal colonization. Infect Immun 84:375–384
2. Meighen EA, Szittner RB (1992) Multiple repetitive elements and organization of the lux operons of luminescent terrestrial bacteria. J Bacteriol 174:5371–5381
3. Bjarnason J, Southward CM, Surette MG (2003) Genomic profiling of iron-responsive genes in *Salmonella enterica* serovar Typhimurium by high-throughput screening of a random promoter library. J Bacteriol 185:4973–4982
4. Meighen EA (1991) Molecular biology of bacterial bioluminescence. Microbiol Rev 55:123–142
5. Gal-Mor O, Gibson DL, Baluta D et al (2008) A novel secretion pathway of *Salmonella enterica* acts as an antivirulence modulator during salmonellosis. PLoS Pathog 4:e1000036
6. Nguyen M, Rizvi J, Hecht G (2015) Expression of enteropathogenic *Escherichia coli* map is significantly different than that of other type III secreted effectors in vivo. Infect Immun 83:130–137
7. Trcek J, Fuchs TM, Trulzsch K (2010) Analysis of *Yersinia enterocolitica* invasin expression in vitro and in vivo using a novel *luxCDABE* reporter system. Microbiology 156:2734–2745