May 15, 2022

Monitoring of *Citrobacter rodentium* shed from infected mice using luminometry and viable counts

Nature Communications

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.rm7vzyo3rlx1/v1

Hannah Read[1], Priyali Patel[1], Siouxsie Wiles[1]

[1]University of Auckland

Siouxsie Wiles
University of Auckland

DOI: https://dx.doi.org/10.17504/protocols.io.rm7vzyo3rlx1/v1

Protocol Citation: Hannah Read, Priyali Patel, Siouxsie Wiles 2022. Monitoring of Citrobacter rodentium shed from infected mice using luminometry and viable counts. **protocols.io https://dx.doi.org/10.17504/protocols.io.rm7vzyo3rlx1/v1**

Manuscript citation:
Read, H. M., Mills, G., Johnson, S., Tsai, P., Dalton, J., Barquist, L., Print, C. G., Patrick, W. M., & Wiles, S. (2016). The in vitro and in vivo effects of constitutive light expression on a bioluminescent strain of the mouse enteropathogen Citrobacter rodentium. PeerJ, 4, e2130. https://doi.org/10.7717/peerj.2130

Protocol status: Working

Created: May 09, 2022

Last Modified: May 15, 2022

Protocol Integer ID: 62298

Keywords: Citrobacter, bioluminescence, ICC180, luminescence, lux, stool, faeces, mouse infection, monitoring of citrobacter rodentium, citrobacter rodentium, viable counts citrobacter rodentium, bioluminescent derivative, lux operon from photorhabdus luminescen, shed from infected mice, gastrointestinal pathogen, laboratory mice, enteropathogenic escherichia coli, infected mice, vivo infection dynamic, photorhabdus luminescen, bacteria present in shed stool, stools of infected animal, using luminometry, bacteria, infected animal, mice, called icc180, infection dynamic, negative bacterium, lux operon, monitoring of citrobacter rodentium shed, citrobacter rodentium shed

Abstract

Citrobacter rodentium is a Gram-negative bacterium which infects laboratory mice in a similar way to how enteropathogenic *Escherichia coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC) infect humans. As a gastrointestinal pathogen, *C. rodentium* is shed in the stools of infected animals. This allows in vivo infection dynamics to be monitored by plating out the bacteria present in shed stools. We routinely use a bioluminescent derivative of *C. rodentium* called ICC180 which contains the lux operon from *Photorhabdus luminescens.* This allows us to monitor infection dynamics using luminometry too.

Guidelines

Experiments involving animals and pathogenic bacteria require ethical and biological safety approval. When planning experiments involving animals, consult the **PREPARE** and **ARRIVE** guidelines.

Luminometry: Luminometers measure light emission using photomultiplier tubes, which convert photons into electrical pulses. Luminometers come in many formats, from a simple manual one-tube luminometer to a computer-controlled micro-titre plate instrument. Detected photons are often displayed as relative light units (RLU) or counts per second. Absolute values of light emission vary greatly from luminometer to luminometer for a given amount of luciferase protein. For this reason, samples from an experiment should be run on the same machine using the same protocol. Also note, when using micro-titre plate instruments, light can spill over from wells containing bright samples to the surrounding wells if using white or clear plates. We routinely use black plates to minimise such spill over.

Materials

	Item	Catalogue number	Supplier
	LB (Lennox) Agar	240110	Fort Richard Laboratories
	Kanamycin	K4000	Sigma-Aldrich
	Phosphate Buffered Saline tablets	P4417	Sigma-Aldrich
	Petridishes - 90mm x 14mm	S9001	medi'Ray NZ
	1.7 mL microcentrifuge tube	AXYGMCT175C	Global Science
	Tweezers		
	Empty 1mL pipette tip boxes		
	Disinfectant		
	Luminometer		
	Vortex		
	Incubator		

Troubleshooting

Before start

Label and weigh individual microfuge tubes. Write the weight on the side of the tube.
Remove inserts from pipette tip boxes

Stool collection

1 Place each mouse in a clean pipette tip box which has had the insert removed.

2 Once a mouse has produced 1-3 individual stools, transfer these to a sterile labelled microfuge tube of known weight using a pair of clean tweezers.

Depending on the mouse strain, stools can become very loose and much more difficult to pick up using tweezers. In these instances, try using a small laboratory spatula instead. We've also observed mice placing their stools up on the walls of the tip box like they are moving them out of the way or making an offering!

3 Transfer the mouse back to its home cage.

4 Clean the pipette tip box and tweezers using an approved disinfectant.

Stool suspension and homogenisation

5 Weigh each tube again to find the weight of the stool.

6 Add PBS to a final concentration of 0.1 g of stool per 1 mL of PBS.

7 Homogenise using a vortex.

Viable counts

8 Allow any large remaining stool particles to sink to the bottom of the tube.

9 Prepare a 10 fold serial dilution of the stool homogenate. This can be done by removing 100 µL of the stool homogenate and adding to 900 µL PBS in a microcentrifuge tube and repeating for as many dilutions as necessary. Alternatively, fill the wells of a 96 well plate with 90 µL of PBS and add 10 µL stool homogenate

to the first well in each column. Then perform serial dilutions down the plate using a multichannel pipette. Make sure to use clean pipette tips for each dilution.

10 Plate 3 aliquots of 25 µL of each dilution onto a dry LB Agar plate containing kanamycin. If the plate is wet, the droplets will roll into each other and you won't be able to count the individual colonies.

11 Incubate plates Overnight at 37 °C and then count the *C. rodentium* colonies present at each dilution.

Luminometry

12 Record the luminescence of the first 1-2 dilutions from the serial dilution of the stool homogenate using a luminometer according to the instrument's instructions.

Depending on the sensitivity of the luminometer, very bright signals can saturate the machine. This is why it is useful to run the first two dilutions at the peak of infection when there are large amounts of bacteria present. If the luminescence reading from the second dilution is not 10 times lower than the first dilution then you will know the first sample was saturated and the reading won't be accurate.

The light from bright samples can also spill over to adjoining wells if using a micro-titre plate luminometer which is why we prefer to use black microtitre plates which reduce such spill over.