phage engineering | Pseudomonas aeruginosa | detection | bioluminescence

# Phage engineering for the detection of *Pseudomonas aeruginosa*

Maria João Costa [1,2#], Luciana Meneses [1,2#], Sílvio B. Santos [1,2], Joana Azeredo [1,2], Diana Priscila Pires [1,2]

1. CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal.
2. LABBELS – Associate Laboratory, Braga, Guimarães, Portugal

Correspondence:
Dr. Diana P Pires: priscilapires@deb.uminho.pt

Maria João Costa and Luciana Meneses contributed equally to this work

*Pseudomonas aeruginosa* is an opportunistic Gram-negative bacterium. Due to its high antibiotic resistance and capacity to adapt and survive in hostile conditions, *P. aeruginosa* is responsible for a wide range of human infections, such as surgical site infections, bacteremia, urinary tract infections, and mostly, pneumonia. In COVID-19 patients, *P. aeruginosa* is a common co-infecting pathogen, associated with increased disease severity and worse clinical outcomes. Considering the slow turnover of conventional diagnostic methods and the problems associated with the molecular and immunogenic methods, this study aimed at assembling a bioluminescence-based reporter phage for the fast and sensitive detection of P. aeruginosa in clinical care.

Phage vB_PaeP_PE3 was genetically engineered using the yeast-based phage engineering platform. The genome of this phage was previously reduced by deleting genes with unknown function, and here, this phage genome was used as a scaffold for the insertion of the NanoLuc® luciferase. The gene encoding NanoLuc was swapped with gene gp55, encoding a hypothetical protein with unknown function. The sensitivity of this phage-based detection system was evaluated through the infection of serial dilutions of *P. aeruginosa* suspensions with the synthetic phage, and subsequent quantification of luminescence (in relative light units, RLU). Our data showed that the reporter phage was able to reliably detect 10^2 CFU in 1 mL of contaminated sample in less than 8 h.

Overall, the NanoLuc-based reporter phage allows for the rapid and sensitive detection and differentiation of viable *P. aeruginosa* cells using a simple protocol, 45 h faster than culture-dependent approaches. Therefore, this phage-based detection system is a promising alternative to the common methods for the accurate detection of *P. aeruginosa* in clinical settings.