

# Engineering a Bacterial Pathogen with an Enhanced Luminescent Reporter for High-Throughput Resistance Assays

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Bioluminescent strains of *Pseudomonas syringae* pathovar (pv.) tomato was developed for a high-throughput resistance assay. There has been an effort to generate a pathogen carrying a luminescent reporter. However, existing strains have never been widely used due to their low efficiency. This project was an attempt to engineer a new strain with a brighter reporter to accommodate a high-throughput analysis. To this end, multiple recombinant reporter genes with three NanoLuc Luciferase (NL 1.1, NL 1.2, & NL 1.3) under a constitutive promoter (proD) were constructed. The NanoLuc Luciferase is a recent luminescence reporter enzyme which has been shown to be at least 100 times brighter than conventional counterparts. New recombinant plasmids were introduced to the genome of *P. syringae* (pst) via a transposon-mediated recombination. These new *Pseudomonas* strains were tested in vitro as well as in vivo, to assess the luminescence efficiency. 96-well plates were used to compare the luminescence among the new strains. Also, *Nicotiana benthamiana* was infiltrated with the new reporter strains. In sum, NanoLuc bacterial strains were bright enough to be detectable under an EM-CCD camera and far brighter than the luxCDABE luciferase strains that have been used by plant pathologists for the last several years. In Conclusion, the development of efficient strains presents a great opportunity to further set up a large-scale experiment in which an unprecedented number of plants would be tested with these pathogen strains.

## Awards Won:

Air Force Research Laboratory on behalf of the United States Air Force: First Award of \$750 in each Intel ISEF Category