Focal Points
Application Note FP-184

Use of Red Firefly Luciferase to Monitor Infiltration of *Nicotiana benthamiana* for *In Planta* Bioluminescence

Tony Sanchez[1], Claire Wiley[2]

[1]UVP LLC, Department of Product Technology, Upland, CA
[2]Pitzer College, Claremont, CA

Introduction

Luciferases are enzymes which are employed by eukaryotes to carry out a variety of behavioral activities ranging from mate selection to warding off predators. These enzymes generate light, a process known as bioluminescence, through an enzymatic reaction in which a luciferase enzyme consumes its substrate and ATP/oxygen to produce a photon of light. The original luciferase enzyme was isolated from the North America firefly, *Photinus pyralis*. Isolation of the luciferase gene, known as *LUC*, and transfection of this gene into the genome of a host at a specific coding region for a protein of interest makes luciferase an important non-invasive genetic reporter, useful for detecting changes in gene expression.

Since the isolation and transfection of the *LUC* gene several decades ago, many additional bioluminescent enzymes have been discovered from a wide range of species. These have been developed into reporters for a multitude of genetic studies, including protein-protein interactions and analysis of gene promoters.

Luciferase catalyzes the oxidative decarboxylation of luciferin through a proposed multi-step process (Figure 1). This decarboxylation reaction within the firefly luciferase system results in the generation of light with a maximum output at 562-570nm and with an incredible degree of efficiency, up to 90% in some cases. Numerous additional enzyme systems have been discovered, culled from other bioluminescent species, and have added to the spectrum of colors available to researchers (Table 1) including luciferases from *Luciola criciata* with an E_{max} in the far red (615 nm) that offer better tissue penetration in vivo.

$$luc + DLu + Mg + ATP \leftrightarrow (luc \cdot DLu\text{-}AMP) + MgPP_i$$

$$(luc \cdot DLu\text{-}AMP) + O_2 \rightarrow (luc \cdot oxyDLu \cdot AMP) + CO_2$$

$$(luc \cdot oxyDLu \cdot AMP) \rightarrow (luc \cdot oxyDLu \cdot AMP) + hv$$

Figure 1. Proposed mechanism for bioluminescence key reactions of luciferases using D-Luciferin. *luc*, luciferase enzyme. *DLu* luciferase substrate D-Luciferin.

Species	Luciferase	Substrate	λ (nm)
Renilla reniformis	Renilla	Coelenterazine	480
Photinus pyralis	Firefly	Luciferin	562-570
Pyrophorus plagiophthalamus	Click Beetle	Several	544-593
Luciola Criciata	Japanese Firefly	D-Luciferin	615

Table 1. List of commonly used bioluminescence enzymes and substrates.

Luciferase reporter systems have been used extensively within *in planta* studies, most notably to study changes in the expression of transgenes[1]. In this study, BLI was applied to visualize the infection of a transgenic organism expressing a red-shifted luciferase into the leaves of a host plant, *Nicotiana benthamiana*.

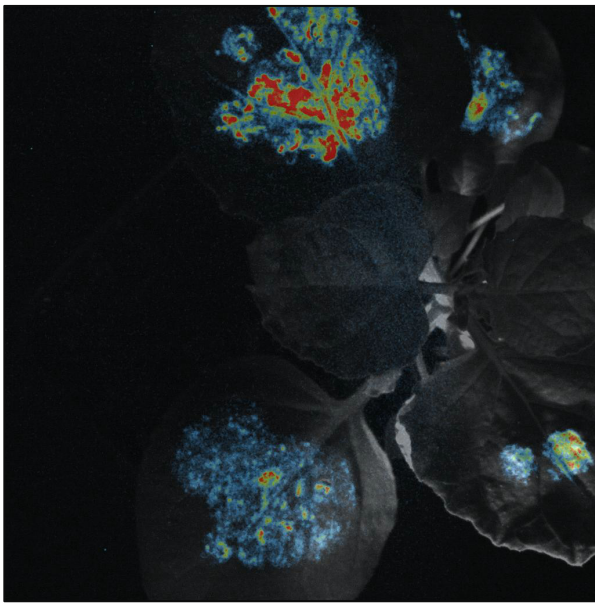

Figure 2. Bioluminescence of *N. benthamiana* leaves infiltrated with *A. tumefaciens* imaged using the iBox Scientia.

Materials & Methods

Experimental Protocol

TMV Inoculation*: N. benthamiana* leaves were infiltrated with *Agrobacterium tumefaciens* (OD_{600} ~1.0) containing pTRBO plasmid with a red firefly luciferase gene insert (Thermo Fisher Scientific, Rockford, IL). When TMV RNA is transcribed, the TMV initiates self-replication and expresses a luciferase protein that is red-shifted with an E_{max} of 615 nm. Five days post infection, the infected leaves were sprayed with a 1mM D-luciferin, 0.01% Tween 80 solution 24, 16 and 6 hours before imaging.

In Vivo Imaging: Image capture was performed using the iBox® Scientia™ equipped with the OptiChemi 610 CCD camera cooled to 55°C below ambient, and an optical system using no filter and a 50mm f/1.2 lens (UVP, LLC, Upland, CA, an Analytik Jena Company) (Figure 3). Bright field and bioluminescence images were captured separately at 1x1 binning. Bright field images using a white light channel were captured first at an exposure time of 150 milliseconds, followed by a bioluminescence channel at an exposure time of 60 seconds.

Image Processing and Analysis: Each bioluminescence image was histogram-adjusted using VisionWorks®LS Acquisition and Analysis Software (UVP, LLC). For visualization of the image, median noise reduction was first applied, followed by histogram adjustment to determine the minimum and maximum grey-scale counts for equalization across all time points. The adjusted image histogram was then normalized, remapped and pseudocolored. To highlight co-localization of bioluminescence at the injection site, both the bright field and bioluminescence images were overlaid.

Results and Conclusion

This study highlights the utility of tracking the expression of a pathogen with bright and stable luciferase genetic reporters using a highly sensitive imaging system. Inoculation of *N. benthamiana* leaves and visualization 5 days post infection reveals a highly distinctive variegation pattern (Figure 2).

Sites of inoculation show the greatest intensity, represented as red areas within the leaf and, with minimal infiltration, tend to be round in shape. More extensive infiltration of *A. tumefaciens* shows a larger pattern of variegation, diffused in nature, with more numerous areas of intense signals.

Characterization of local infection within the host species can be incredibly useful, particularly with pathogenic organisms of economically important agricultural crops. Linking the genetic reporter luciferase to a protein that enables virulence allows for visualization of infection and a better understanding the mechanism of disease.

Equally important to a stable and bright luciferase system is the use of an imaging system that is able to detect the low light output of luciferase enzyme systems. The iBox Scientia is the ideal imaging system for *in planta* luciferase due to its highly sensitive camera and optics which minimize background for long exposures, often required for *in planta* studies.

Figure 3. iBox Scientia Imaging System

[1] Van Leeuwen, Wessel, et al. "The use of the luciferase reporter system for *in planta* gene expression studies." *Plant Molecular Biology Reporter* 18.2 (2000): 143-144.