

SCIENCE IMAGING SYSTEMS

Application Note No. 23

Gene Expression Analysis Using Luciferase as Reporter Gene

LAS-3000

Foreword

Gene expression analysis using luciferase, the substance that gives fireflies their glow, is steadily gaining acceptance. Since the feeble light of bioluminescence is very difficult to detect with an ordinary system, it was not until the advent of the high-sensitivity cooled CCD system that methods using this weak light could be put into practice. In this Application Note, Dr. Yoshida from the Science Laboratory, Osaka City University explains an example of gene expression analysis performed by introducing genes fused with luciferase.

Detection was performed using the LAS-3000 cooled CCD system equipped with a super CCD that is presented in Application Note No. 22.

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Summary

- A gene obtained by fusing luciferase as reporter gene to the 35S promoter of cauliflower mosaic virus was introduced into a plant.
- The plant was sprayed with luciferin and the gene expression was imaged *in vivo* using the LAS-3000.
- The Colony analysis program was used to determine the amount of expression from the image.

1 Introduction

A method commonly used in gene expression analysis is to detect the expression of a reporter gene attached downstream of the transfer regulating region (promoter, enhancer etc.) of the gene of interest. The main reporter genes used in plant research are luciferase (LUC) gene, β -glucuronidase (GUS) gene, and green fluorescent protein (GFP) gene. Among these, luciferase is known for its high sensitivity and excellent quantifiability. Another advantage of luciferase is that its substrate, luciferin, has little toxicity to plants and can be readily absorbed through the plant surface. This facilitates *in vivo* observation of gene expression. In the present study, luminescence occurring during oxidation of luciferin by luciferase was imaged for quantification by a cooled CCD camera. A plasmid having an LUC gene attached downstream of a cauliflower mosaic virus (CaMV) 35S promoter and capable of expressing a high-level LUC gene was introduced directly into the plant body using a particle gun. The luciferase luminescence was found to be quantifiable after imaging with the LAS-3000 (Fuji Photo Film Co., Ltd.).

Terminology

Reporter gene

A common practice in gene expression analysis is to attach an easily detectable and quantifiable protein gene such as luciferase (LUC) or β -glucuronidase (GUS) downstream of the gene of interest and then examine the expression of the attached gene. This gene, which serves as an indicator, is called a "reporter gene."

β -glucuronidase (GUS)

GUS is an enzyme that produces D-glucuronic acid by hydrolyzing β -glucuronide. The enzyme activity is determined by detecting fluorescence or color formation. In fluorescence detection, fluorescence produced by using 4-MUG (4-methylumbelliferyl- β -D-glucuronide) as substrate is measured with a fluorescence spectrophotometer. In color formation detection, a sparingly water-soluble blue pigment produced by enzyme reaction using X-Glus (5-bromo-6-chloro-3-indolyl- β -D-glucuronide) is observed.

Green fluorescent protein (GFP)

GFP is the general term for the green fluorescent proteins found in luminous jellyfish. These proteins absorb long-wavelength UV or blue light and emit green fluorescence. Since generation of fluorescence does not require addition of a cofactor such as substrate or Mg^{2+} , GFP fluorescence can be observed without pretreatment.

Cauliflower mosaic virus (CaMV) 35S promoter

CaMV is the first plant virus demonstrated to contain DNA as genetic material. It infects plants belonging to the Brassicaceae family. CaMV is a circular virus (diameter: 50 nm) whose genome consists of open circular double stranded DNA. The mRNA transferred from the double stranded DNA is of two types: 35S and 19S. 35S is a powerful promoter that controls CaMV gene expression and is capable of strongly expressing a specific gene within a plant. This has led to wide use of a method that involves introducing into a plant a fused gene obtained by combining a CaMV 35S promoter DNA fragment and the subject gene.

CloseUp**What is luciferase (LUC)?**

Luciferase is the general term for enzymes that catalyze bioluminescence in fireflies and some other creatures. LUC generates light by oxidizing luciferin in the presence of ATP. Its substrate, luciferin, is water soluble and is therefore easily ingested by cells. LUC can produce its fluorescence reaction inside living cells because neither it nor its reaction product is toxic to living organisms.

How does luciferase produce luminescence?

2 Test Procedure

■ Gene introduction

- 1) Leaves were cut from a plant^{*1} and placed on an MS culture medium plate containing 0.3% Gelrite. A particle gun was used to introduce a plasmid having an LUC gene attached downstream of a CaMV 35S promoter. (Fig. 1)

*1 Five-day old morning glory cotyledons were used in the test.



Fig.1

Fig. 1 Introduction of genes using particle gun

- 2) The Petri dish containing the culture medium plate was covered and incubation was conducted at 23 °C and RH 50% for 19-20 hr.

Experimental Tip

Although LUS activity can be ascertained from immediately after introduction, measurement is usually done after 12-24 hours. The luciferase gene is expressed and the enzyme activity becomes strongest.

■ Luciferin treatment

- 1) The leaves in the Petri dish were treated by uniformly spraying their surfaces with a 3 mM aqueous solution of luciferin (containing 0.01% (v/v) TritonX-100).
- 2) The leaves were left standing for 20 min following the treatment.

Experimental Tip

1 mM, 3 mM, and 5 mM aqueous solutions of luciferin were tried. A 3 mM aqueous solution was used because the luminescence was found to be somewhat weak at a concentration of 1 mM. Instead of spraying, it is possible to apply a small amount of the luciferin aqueous solution with a brush.

■ Reading the luminescence image

- 1) The Petri dish was placed in the LAS-3000 dark box, the detection conditions were set (see Fig. 2 for the reading conditions), and the focus was adjusted.
- 2) The dark box door was closed and, after a wait of 5 minutes to allow attenuation of fluorescence attributable to chloroplasts^{*2}, exposure was carried out for 10 min.

*2 Chlorophyll excited by light returns to the base level state by emitting longer wavelength fluorescence. The fluorescence rapidly reduces in the dark.

Experimental Tip

Care should be taken to avoid dark box temperature fluctuation because LUC activity is strongly affected by temperature.

The read image was saved. (Fig. 2)

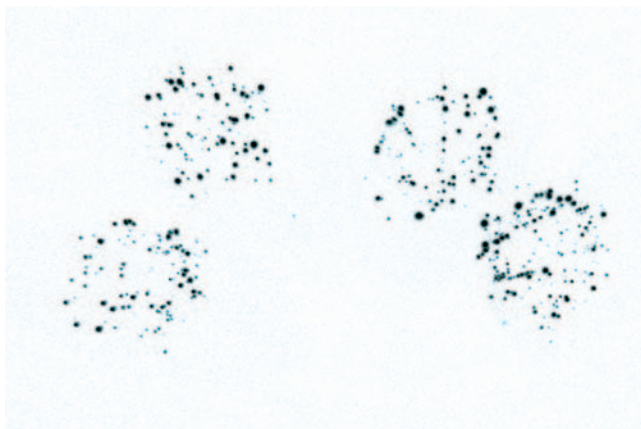


Fig. 2

Fig. 2 Detection by LAS-3000

Reading conditions:
 Method: Chemiluminescence
 Tray position: 2
 Sensitivity: Standard
 Exposure time: 10 min

3 Analysis results

Image analysis using analysis software

- 1) The saved image was opened after launching the Colony analysis software*¹ (see pages 6-7).
- 2) The expression spots were extracted.

*1 An image analysis software included in ScienceLab2003 (for Windows) from Fuji Photo Film Co., Ltd.

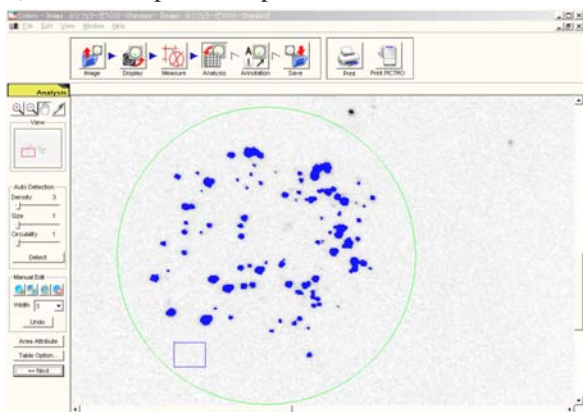


Fig. 3

Fig. 3 Expression extraction using Colony software

- 3) The extracted spots were quantified.

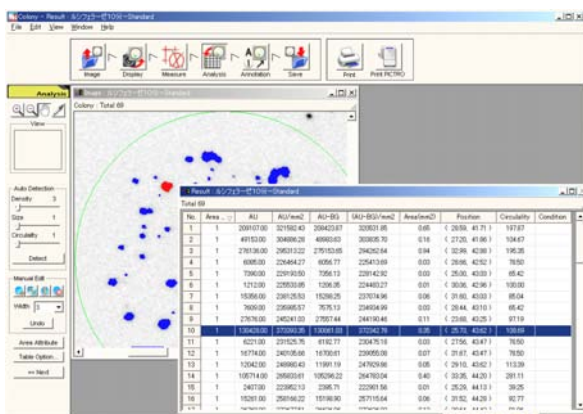


Fig. 4

Fig. 4 Quantification results

- 4) The quantification results were converted to a text file and opened in Excel.
- 5) The total value of the expression was calculated.

4 Conclusion

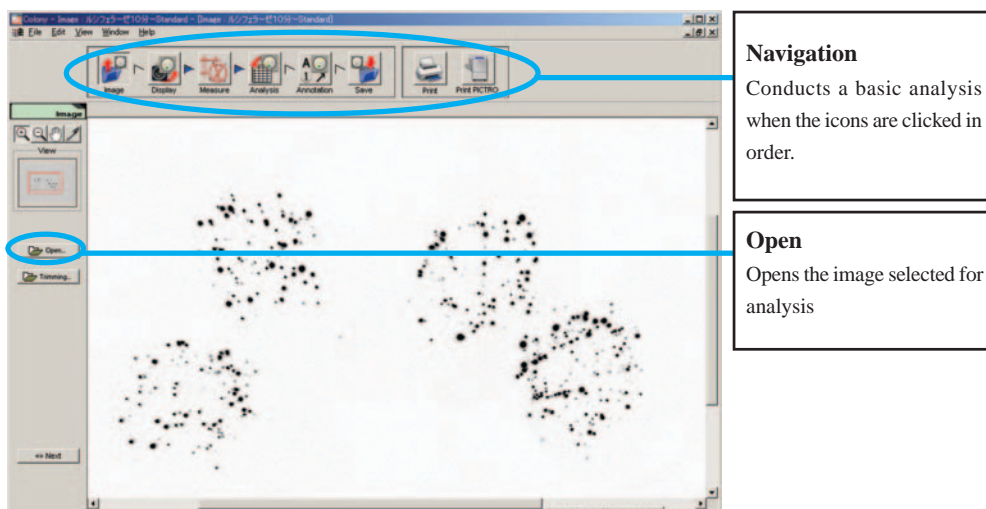
The LAS-3000 was used for gene expression analysis employing luciferase as the reporter gene and the amount of expression was quantified using the Colony analysis software. Thanks to its relatively short half-life *in vivo* state, luciferase is ideal for expression analysis that follows time-course changes, as in the analysis of the light induction and circadian rhythm of the introduced gene. Imaging/quantification of luciferase luminescence using the LAS-3000 can therefore be considered an effective method for this type of gene expression analysis. It is planned to pursue gene expression analysis by using LAS-3000 to quantify gene expression and converting the expression amounts into numerical values.

CloseUp

Colony analysis software

Colony is a software that automatically extracts, counts and quantifies colonies or expression spots on a Petri dish or plate. Analysis is easy to conduct simply by following the Navigation procedures, which are explained below using Dr. Yoshida's images as an example.

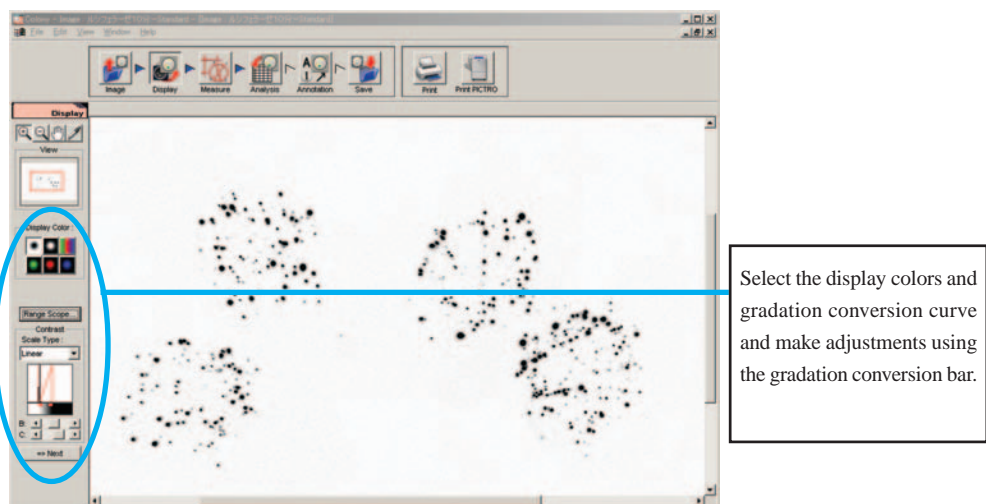
(1) Open the image. <Image mode>



Navigation
Conducts a basic analysis when the icons are clicked in order.

Open
Opens the image selected for analysis

(2) Adjust the gradation to get an easy-to-view image. <Display mode>



Select the display colors and gradation conversion curve and make adjustments using the gradation conversion bar.

(3) Create extraction region and background. <Measure mode>

Extraction region

Create extraction region
Not only circular but also elliptical settings are possible. The interior of the region can be partitioned with dividing lines.

Create background region
The plate background can be subtracted.

Background region

(4) Set extraction conditions and click the Detect button. <Analysis mode>

Extracted colony
Displayed in blue.

Add, correct or delete extracted colonies

Extraction conditions

The density, size and circularity of extracted colonies can be set as conditions. Colonies satisfying all three conditions are extracted.

Density : Setting a smaller number extracts lighter colonies. The minimum and the maximum densities within the extraction region are assigned to parameters 0 and 100 respectively.

Size : Setting a smaller number extracts colonies of smaller area.

Circularity : This is a parameter for setting how near perfectly circular a colony must be to be extracted. Setting a larger number extracts more circular colonies. The range of settings is 0 to 100.

5 Reference

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- 4) Muramoto, T. and Aoyama, T., Making use of reporter genes, *Model Plant LaboManual*, Springer-Verlag Tokyo, 194-203 (2000) (in Japanese)
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