Information and Specifications: Xenogen IVIS 50 Bioluminescent System

Background

Bioluminescence is the production and emission of light by a living organism as the result of a chemical reaction during which chemical energy is converted to light energy. This chemo-luminescence reaction occurs when the substrate, e.g. luciferin, is oxidized by the enzyme luciferase to produce an oxidized substrate, e.g. oxyluciferin, and energy in the form of light. This process is different from fluorescence where an exogenous source of light is absorbed then remitted as another wavelength of light. Because there is no exogenous light source with bioluminescence, there is no autofluorescence that can obscure low-level signals. This results in higher sensitivity detection compared to fluorescence. However, because it is a chemical reaction, the substrate must be injected just prior to each imaging session and light production will only last as long as the reaction continues (approximately 1-30 min depending on amount of substrate injected).

Bioluminescence imaging is a high-sensitivity, low-noise, non-invasive technique used for visualizing, tracking, and monitoring specific cellular and genetic activity in an animal. This specificity comes from the 'tagging' of the target gene or cells with Firefly or *Renilla* luciferase The luciferase enzyme used is encoded by the luc gene (F*luc* in Firefly luciferase and R*luc* in *Renilla* luciferase). Both luciferase proteins are monomers that do not require any posttranslational modifications; they are available as a mature enzyme directly upon translation from their respective mRNAs. Firefly luciferase is a 62 kDa monomer and *Renilla* luciferase is a 36 kDa monomer. The kinetics of these two enzymes varies significantly. Firefly luciferase has a $\mathbf{K_m}$ of 180 and a $\mathbf{V_{max}}$ of 101 molecules luciferin/s/mole enzyme whereas *Renilla* luciferase has a $\mathbf{K_m}$ of 2.9 and a $\mathbf{V_{max}}$ of 2.0 e^{24} molecules coelenterazine/s/mole enzyme. Hence, the *Renilla* luciferase reaction will occur more rapidly than the Firefly luciferase reaction. Due to this kinetic difference, the route of administration and dose differs between the two (refer to table 1). It should be noted that both reactions require oxygen to proceed and Firefly luciferase also requires ATP and magnesium. This is an important consideration as ATP is crucial for cell viability. Therefore, when ATP is a potential variable, coelenterazine with *Renilla* luciferase would be the suggested option for experimentation.

The luciferase gene is found in many distinct classes of bioluminescence derived through separate evolutionary histories. Although these classes are widely divergent in their chemical properties, they all undergo the same basic chemical reaction, namely an oxidation-reduction reaction in which a substrate is oxidized by luciferase and luciferase is reduced (refer to figure 1). 98% of the energy released in this oxidation-reduction reaction is in the form of light. The remaining 2 % dissipates as heat. Therefore, this exergonic process is referred to as a cold reaction. When the genes or cells are activated, they emit light, which the system can passively detect and can then record the resulting images.

The emission spectrum of the emitted light has a major impact on whether it reaches the surface of the animal and is thus detectable. The peak emission wavelength for Firefly luciferase is 560 nm and for *Renilla* luciferase it is 475 nm (refer to figure 2). A typical bioluminescence image can take 1 to 5 minutes to acquire. Using the maximum field of view, up to three whole mice can be imaged simultaneously, while another three mice can be anesthetized in an external induction chamber. Using this staggered system, large sets of animals can be processed in a relatively short period of time.

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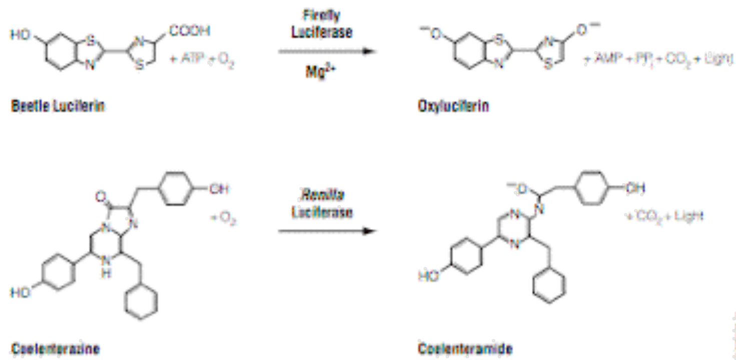
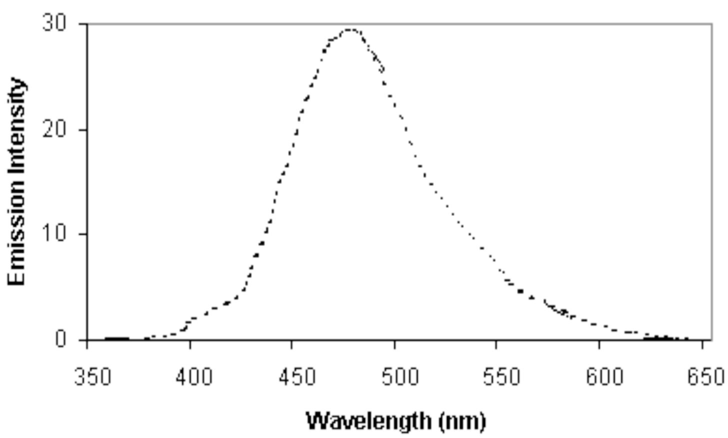


Figure 1: Exergonic, oxidation-reduction reaction between Firefly luciferase and luciferin (top) and Renilla luciferase and coelenterazine (bottom) to produce light. O2 is required for both reactions, but Mg 2+ and ATP are only required for the Firefly luciferase & luciferin reaction.

Coelenterazine Emission Spectrum



Luciferin Emission Spectrum

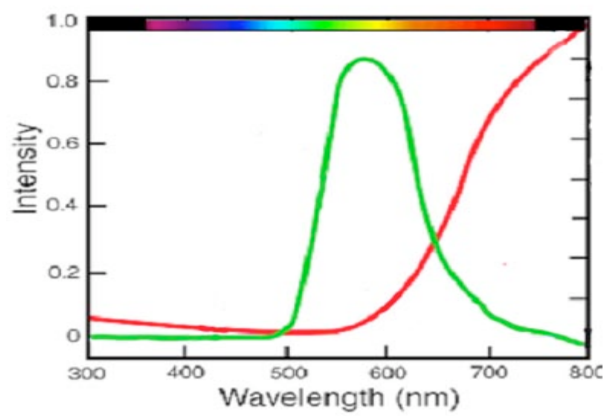


Figure 2: Peak Fluorescence of Coelenterazine (left) and Luciferin (right). In the Luciferin Emission Illustration, the green line denotes luciferin's peak emission wavelength and the red line represents the attenuation tissue encounters at a depth of 1cm . This is dramatically decreased in the near-infrared region (700-1000 nm).

SMALL ANIMAL IMAGING APPLICATIONS

One major use for bioluminescence is to combine a promoter sequence with the expression of luciferase to non-invasively quantify promoter activity in vivo. There is a difference between transient versus stable transduction with respect to introducing the promoter sequence combined to luciferase expression into a cell line. 24-48 hours after transient transduction, a subject may be ready for imaging. This effect is short term. However, in longitudinal studies, a stable transduction would be necessary which would allow the expression at any time and be consistent throughout the genome. An example of a Luciferase Reporter Vector (Panomics) is illustrated in Figure 3. The use of reporter genes has been mostly to investigate the function of cis-acting genetic elements such as promoters and enhancers. In experiments, deletions or mutations are made in a promoter region, and their consequential effects on coupled expression of a reporter gene are then quantified. This is often achieved by using viral promoters that are fused to luciferase as transgenes in animal models allowing the external observation of gene expression at the surface of the animal as well as deep tissue.

However, the broader aspect of gene expression entails much more than transcription alone, and reporter genes can also be used to study these other cellular physiological events.

Once the animal has been transfected with the vector. A bioluminescent experiment can be conducted 24-48 hours later. The expression of the gene can be controlled by the amount of substrate given. This is demonstrated by an increase in signal. Another method for introducing the luciferase reporter is through electroporation.

Another application of bioluminescence is to track the number and/or location of cells inside a subject. Once the target cells have been tagged with the appropriate luminescent marker, firefly or *Renilla* luciferase, the cells can be tracked over time by injecting and visualizing the marker's substrate, either luciferin (for firefly) or coelenterazine (for *Renilla* luciferase). This technique, for instance, could be used to monitor the

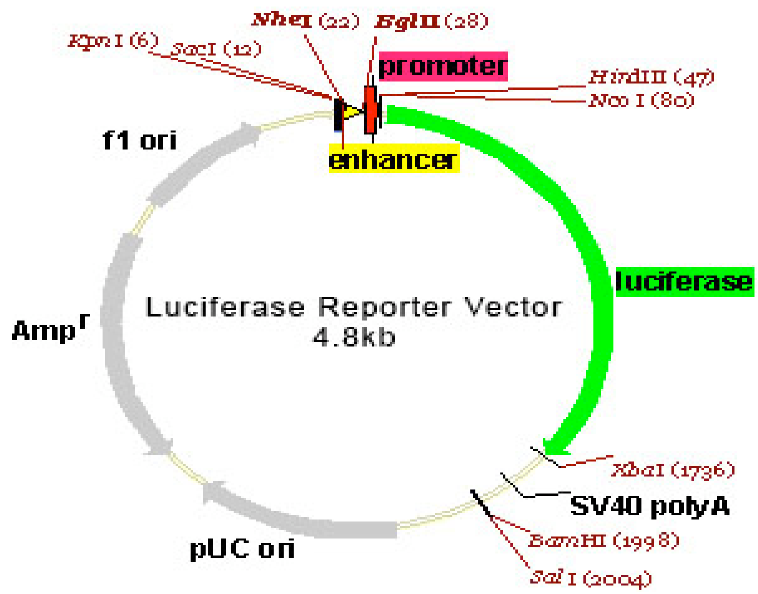


Figure 3: Luciferase Reporter Vector (Panomics)

growth and metastasis of tumors.

Some other applications include identifying interacting pairs of proteins in vivo using a system known as the two-hybrid system (Fields et al, 1989). The two proteins are combined as fusion partners – one is fused with a specific DNA binding domain, the other is fused with a transcriptional activation domain. The physical interaction of the two fusion partners is necessary for the functional activation of a reporter gene driven by a basal promoter and the DNA motif recognized by the DNA binding protein. Another way to monitor protein-protein interactions is by energy transfer. When the fusion protein is made using the bioluminescent *Renilla* luciferase and another protein fused with a fluorescent molecule, interaction of the two fusion proteins results in energy transfer from the bioluminescent molecule to the fluorescent molecule, with a simultaneously change from blue to green light (Angers et al., 2000).

Table 1 : Comparative information of luciferase substrates and enzymes		
Common Name	Firefly	Sea Pansy
Species	*Photinus pyralis*	*Renilla reniformis*
Gene Name	Fluc	Rluc
Enzyme Molecular Weight	62 kD	36 kD
Substrate	Luciferin	Coelenterazine
Dose	150 mg/kg	0.7 mg/kg
Cost	$600 per 1 g	$190 per 1 mg
Effective Cost (per 25g mouse)	0.025 kg * 150 > 3.75 mg = $ 2.25	0.025 kg * .7 > .02 mg = $3.33
Route of Administration	Intraperitoneal	Intravenous
Peak Wavelength	560 nm	475 nm
V_{max}	101 moleculesluciferin/s/mol enzyme	$2.0e^{24}$molecules coelenterazine/s/mol enzyme
K_m	181 +/- 2.0 μM with ATP	2.9 +/- 1.0 μM

INSTRUMENT SPECIFICATIONS

Hardware

Field of View (width x length):	5 cm X 5 cm to 12 cm X 12 cm (variable zoom)
Resolution:	50 to 400 µm (based on zoom lens position)
Sensitivity:	100 photons/s/cm^2/sr
Scan Time:	1 to 5 min
Reconstruction Time:	1 to 30 sec
Scans needed for 1 mouse (Nose to Rump):	1 scan (at farthest position) to 3 scans (at nearest position)
Maximum whole Mice per Scan	3

The Xenogen IVIS 50 (Lumina) consists of a CCD camera, an imaging camera, optical filter wheel, high reflectance hemisphere and an acquisition computer. The CCD camera, manufactured by Andor Corporation, is a scientific grade, cooled, back-thinned, back-illuminated and has a large format. The Imaging Chamber is Xenogen's unique and highly specialized light tight unit consisting of the housing chamber, a heated moveable platform, a focusing lens system with f/stop control, a filter wheel and sample illumination LEDS. All of the components are controlled by the computer used for the acquisition. The Field of View has 4 settings, A, B, C and D, corresponding to 4 to 12 cm respectively.

Software

Living Image® Software is the image acquisition, and analysis product from Xenogen. It provides a straightforward interface for imaging and data collection. The camera control panel sets and displays all imaging parameters, from exposure time to lens aperture and focus. The process is designed to minimize setup time and maximize throughput.

Operating System:	Windows XP, 2000, NT and Mac OS X
Supported File Format:	TIFF, BMP, PNG
Image Data Types:	32-bit floating-point and 32-bit RGB color
Average File Size:	1 Mb to 5 Mb
Possible Analysis Techniques:	Measure area, mean, standard deviation, min and max of selection or entire image. Generate histograms and profile plots.

Glossary of Terms

1) **Active site** – Specific site on an enzyme where catalysis occurs.
2) **ATP -** adenosine triphosphate, the energy storing molecule of all living organisms.
3) **Autofluorescence** – Natural fluorescence emitted from the subject
4) **Bioluminescence** – The production and emission of light by a living organism as the result of a chemical reaction during which chemical energy is converted to light energy.
5) **CCD** – Charge Coupled Device
6) **Cold reaction** – An exothermic process in which very little heat is released.
7) **Electroporation** – Application of an external electric field to significantly increase the electrical conductivity and permeability of the cell plasma membrane and insert genetic material.
8) **Endothermic reaction** – A chemical reaction that requires energy.
9) **Energy** – The ability to do work or transfer heat.
10) **Enzyme** – A protein molecule that acts to catalyze specific biochemical reactions.
11) **Exothermic reaction** – A chemical reaction that gives off energy.
12) **Firefly** – The common name for the beetle, firefly luciferase, that produces fluorescent light.
13) **Fluorescence** – The emission of radiation, especially of visible light, by a substance during exposure to external radiation.
14) **Luciferase** – The enzyme that catalyzes the oxidation of the luciferin substrate through an oxidation-reduction reaction. It is reduced in the oxidation-reduction reaction.
15) **Luciiferin** – The generic term for light emitting substrates. Luciferin is oxidized in the oxidation-reduction reaction with luciferase.
16) **Substrate** – A substance that undergoes a reaction at the active site in an enzyme.

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