



Bioluminescent and Fluorescent Imaging Vectors Cat. # BLIVxxx

User Manual

ver. 1-022212

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List of Components

Each vial contains 10 µg plasmid DNA in TE buffer.

Vials are shipped at room temperature or on blue ice/dry ice and should be stored at -20°C upon receipt. Properly stored samples are stable for 1 year from the date received.

I. Introduction

Molecular imaging techniques to visualize cell kinetics in small animals have resulted in an explosion in the knowledge of tumor, infectious disease, and stem cell biology. The sensitivity and accuracy of *in vitro* and *in vivo* cell tracing offers several advantages over traditional methods through animal sacrificing and histological analysis. Molecular imaging, for example, is normally non-invasive and allows for quantitatively assessing tumor growth and the effects of therapy over time. Over the past decade, significant advances have been made in molecular imaging technology, which include bioluminescence imaging (BLI), fluorescence imaging (FLI), and enzyme-based positron emission tomography (PET).

Fundamentals of Molecular Imaging

Molecular imaging requires the selection of a molecular probe and imaging system. Protein probes are preferred, because proteins are more abundant within a cell. Normally, the level of proteins can reach 0.01 to 1 million as compared with 1 to 2 DNA or 10 to 1000 RNA, which contributes to the imaging sensitivity.

BLI uses light generated from a luciferase enzyme-substrate and an ultrasensitive cooled charge-coupled camera for signal detection. BLI may have the highest imaging sensitivity (even down to the one cell level), is high-throughput, easy-to-use system, and low cost.

FLI uses red/green fluorescent protein (RFP or GFP) as a probe. Signal generation is achieved by exciting the fluorescent proteins at a given light wavelength and detection light emission at another wavelength with a charge-coupled camera. Compared to BLI, FLI is less sensitive with higher background. In most cases, FLI is used for live imaging of shallow tissues. RFP and GFP give more histological information and can be readily used for cell sorting. Now, FLI is normally coupled with BLI to provide an additional means for cell selection, sorting, thus gaining more histological information.

Enzyme-based positron emission tomography uses HSV1-tk (Thymidine Kinase, TK) as a probe. Signal generation is achieved by the retention of radioisotope labeled chemicals for SPECT or

PET imaging. This modality can be used for large animals or humans with detailed 3-dimensional capability but, it is more expensive to use this system. HSV1-tk, however can serve as therapeutic transgene (e.g. a suicide gene) because when cells express HSV1-tk, expression can be turned off by delivery of a pharmacological dose of the antiviral prodrugs, Ganciclovir or Penciclovir.

Double or Triple Reporters combine the use of different imaging modalities. Each of these above modalities has unique applications, advantages, and limitations that can be complementary to other modalities. For example, a cell-based technique (with GFP or RFP) has limitations for whole body *in vivo* imaging studies, whereas techniques involved in imaging at the organ level (with Luciferase or TK) do not have the resolution power for imaging gene expression at the cellular level. Among the whole body imaging modalities, radionuclide-based techniques (with TK) have a high sensitivity, spatial resolution, and are tomographic in nature, but are limited by the higher cost (especially for PET). In contrast, optical imaging using fluorescence and bioluminescence represent a low cost and quick alternative for real-time analysis in small animal models but may be limited by depth penetration for larger animals and humans. To overcome the limitations of each modality, a double or triple reporter combination can be used. This includes a combination of GFP/RFP with luciferase or a combination of GFP/RFP with luciferase and TK genes. These genes can be co-expressed as individual proteins or as fusion proteins.

Vector delivery systems for Molecular Imaging

Live animal imaging is becoming an increasingly common technique for accurate and quantitative assessment of tumor and stem cell biology in a spatial and temporal manner. The first step for this procedure is to introduce molecular probes into your traced cells. This approach involves transfecting/transducing cells with a reporter gene or a combination of reporter genes. Lentiviral gene delivery system is widely accepted for this purpose because of its high transduction efficiency broad cellular tropism. Minicircles (small, episomal DNA vectors) are also a good choice for *in vivo* experiments because of their long-term, but non-integrative expression, and easy delivery through tail-vein injection.

SBI BLI Technology

The SBI BLIVs are built on two vector backbones. The first is upon an HIV-based lentiviral expression system which enables broad tropism for delivery, stable expression via integration, robust synthesis, and expression by using appropriate promoter elements. The second vector backbone is a minicircle backbone. For a full description of minicircle technology, please see the SBI website at:

<http://www.systembio.com/minicircle-dna-vectors/overview>

To ensure the expression of these constructs in a variety of cell types, constructs are available with three extensively-tested promoters: CMV, UBC and MSCV. The Cytomegalovirus promoter (CMV)-driven constructs can be used for most of cell types. Murine stem cell virus promoter (MSCV) is useful for stem cells and hematopoietic cell types, where CMV promoter may be a concern of inactivation. In addition, the UBC/EF1 promoter is commonly used for imaging stem cells and cancer stem cells, because of their relative resistance to inactivation in those cell types.

Firefly luciferase is chosen for Bioluminescence imaging (BLI), because firefly luciferase has very low background and its substrate D-luciferin has very high bioavailability. At times, the sensitivity may reach single cell level; therefore firefly luciferase is believed to be a better choice when they are used as *in vivo* imagers. In addition, luciferase expression and bioluminescence does not affect tumor cell growth *in vitro* or *in vivo*.

Fluorescent imaging (FLI) is based on fluorescent proteins, such as RFP and GFP. In general, the light emission of RFP has a better tissue-penetration than that of GFP and thus better imaging choice. However, either RFP or GFP tends to have higher background and the sensitivity is lower than that of luciferase. However, the use of RFP/GFP offers excellent histological information if combined with BLI. Both RFP and GFP are fixation-resistant and can provide quality histological information about the traced cells in relation to host cells.

SBI provides a good combination of FLI and BLI to meet your experimental needs. The rule-of-thumb for choosing the right format shall be empirically determined based on successful applications in similar situations. A combination of GFP (best for

histology) and firefly luciferase (best for *in vivo* imaging) is highly recommended for initial experimentation.

II. Available BLIV Vectors

Choice of your BLIV vectors

Lentibased BLIV vectors: The lenti-based BLI vectors can be used as regular plasmid DNA with a transient transfection protocol for preliminary experiments. For high efficient gene delivery, they can be packaged into pseudoviruses and used to infect most cell types both *in vitro* and *in vivo*. As viruses, lenti-based vectors have nearly 100% integration so they are suitable for establishing stable cell lines and high-throughput applications.

If you need a high efficient non-viral gene delivery system, minicircle technology provides an innovative means to effectively deliver and express genes epichromosomally. These minicircle DNAs express your gene of interest in tissue for much longer and at much higher levels as compared to regular plasmid.

Promoter choices

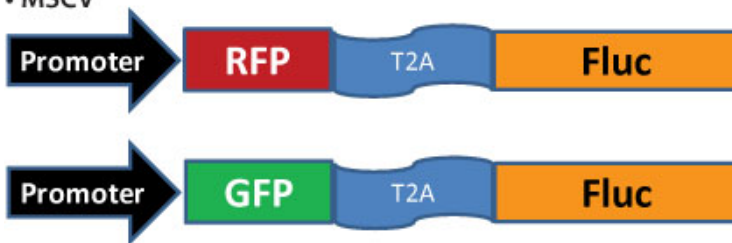
- EF1 alpha
- CMV
- Ubc
- MSCV

Reporter combos

- GFP-T2A-Luciferase
- RFP-T2A-Luciferase

Vector options

- Lentivector
- Minicircle DNA



For a full listing of the available BLIV vectors, please visit the SBI website.

Lenti-based Vector Components

The components of the pBLIV lenti-vectors are displayed in the table below.

Hybrid RSV-5'LTR promoter	For HIV-based vectors. Provides a high level of expression of full-length pseudoviral constructs in 293 producer cells.
cPPT, GAG, LTRs	Genetic elements necessary for the packaging, transduction, and stable integration of the viral expression construct into genomic DNA.
CMV/MSCV/UBC/EF1	Promoter that drives expression of the GFP-fusion protein in the construct. The MSCV promoter is useful for cells that are difficult to infect or transfect such as stem cells and hematopoietic cells. The CMV promoter is useful for easy-to-infect cells.
WPRE element	Enhances stability and translation of the lentivector-driven transcripts.
5' and 3' Δ -LTR	Self-inactivating LTR that enables integration into the host genome.
pUC origin	Ensures high copy replication and maintenance of the plasmid in <i>E.coli</i> cells.
Ampicillin resistance	Used for selection in <i>E. coli</i> cells.
SV40 origin	Provides stable propagation of the lentiviral plasmid in 293TN producer cells.
SV40 polyadenylation signal	Enables efficient termination of transcription and processing of recombinant transcripts.

RFP, GFP, and Firefly Luciferase Characteristics

SBI's GFP is a novel natural green monomeric GFP-like protein derived from copepod (*Pontellina sp.*). The copGFP has optimized human codons for a high level of expression of the fluorescent protein. CopGFP is a non-toxic, non-aggregating protein with fast protein maturation, high stability at a wide range of pH (pH4-12), and does not require any additional cofactors or substrates. The copGFP protein has very bright fluorescence that exceeds at least 1.3 times the brightness of EGFP, the widely used *Aequorea victoria* GFP mutant. The copGFP protein emits green fluorescence with the following characteristics:

excitation wavelength max= 482 nm
emission wavelength max = 502 nm

Due to its exceptional properties, copGFP is an excellent fluorescent marker that can be used instead of EGFP for monitoring molecular dynamics.

SBI's RFP is monomeric and with optimized human codons for a high level of expression. The RFP protein emits red fluorescence with the following characteristics:

excitation wavelength max = 558 nm
emission wavelength max = 605 nm

Firefly luciferase uses D-luciferin as its substrate. Because D-luciferin has a very high bioavailability, firefly luciferase is still the most desirable molecule for *in vivo* imaging.

III. Protocol

A. Transfection of BLIV Vector DNA into Target Cells

The BLIV vector DNAs can be transfected into target cells for use in pilot experiments or for use in cells that are easy to transfect. We recommend using SBI's PureFection™ nanotechnology-based transfection reagent. For difficult cells, other commercially-available transfection reagents may be used.

B. Transduction of BLIV Lenti-viruses into Target Cells

For cells that are more difficult to transfect, such as stem cells, primary cells, or hematopoietic cells, or to create stable tracer cell lines, we recommend packaging the lenti-based BLIV vector DNAs into virus and then transducing the target cells with the viruses. For a protocol and list of necessary supplies and reagents, please refer to the Lentivector Expression Systems manual:

http://www.systembio.com/downloads/web_manual_lentivector_exp_sys_071510.pdf

C. Minicircle BLI

Minicircles (MC) are circular DNA elements that no longer contain antibiotic resistance markers or the bacterial origin of replication. These small vectors can be used as effective and non-integrating gene delivery vectors without the risk of immunogenic responses that can be caused by the bacterial backbone associated with regular plasmids.

Production of minicircles requires a special parental plasmid and an engineered *E. coli* strain that allows both propagation of the parental plasmid and the production of the minicircles. Minicircles are conditionally generated by an expression of inducible Φ C31. For a protocol and list of necessary supplies and reagents, please refer to the Minicircle manual:

http://www.systembio.com/downloads/Manual_minicircle_ver1.pdf

D. Use D-Luciferin substrate for *in vivo* and *in vitro* experimentation

D-luciferin is the substrate for firefly luciferase. Traditional dosing for *in vivo* imaging is recommended at 150mg/kg via intraperitoneal administration. This concentration works well across species (mice, rat, rabbit, monkey, etc). In most cases, a lower dose may also work. The rule-of-thumb is consistency within an experiment. At higher dosage, such as 450mg/kg, no toxicity has been observed, but the benefit in signal intensity over 300 mg/kg is minimal.

Procedure (*in vivo*):

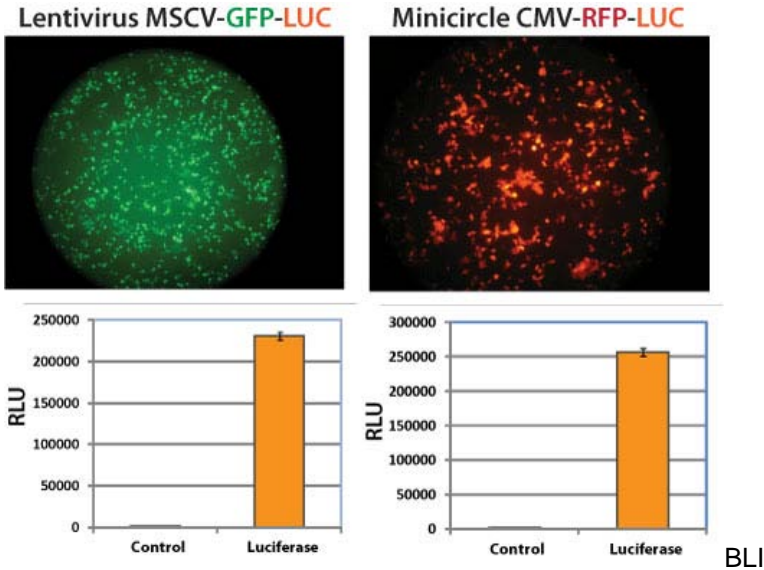
1. Thaw injection-ready D-Luciferin substrate solution at room temperature
2. 10-15 minutes before imaging, the D-Luciferin substrate solution (~ 100 ul/mouse) should be injected intra-peritoneally. A kinetic study of Luciferin may be performed for each animal model to determine peak signal time.

Procedure (*in vitro*):

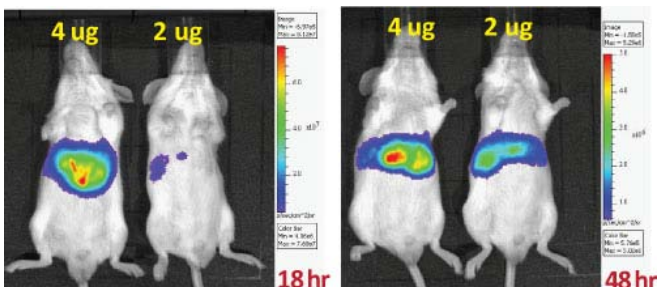
1. Thaw injection-ready D- Luciferin substrate solution (stock, 30 mg/ml) at room temperature.
2. Add stock solution of Luciferin to pre-warmed tissue culture medium to a final concentration of D-Luciferin 150 µg/mL solution (prepared in step 1, as 200X) to culture cell medium immediately before imaging or luciferase assay. The signal is stable for at least 20 min. at room temperature and/or 37 °C.
3. Alternatively, the D-Luciferin imaging medium can be prepared using either complete medium, for example DMEM with 10% FBS or DMEM only.

IV. BLIV Vector Validation Data

Use either BLIV lentiviral vectors to generate stable cell lines or utilize Minicircle vectors to make nonviral and long-lasting (several weeks) reporter cells.



Vectors can be introduced into animal models directly through hydrodynamic tail vein injections. Mice were injected with either 2 ug or 4 ug Minicircle DNA GFP+Luciferase BLI vectors. The mice were injected peritoneally with SBI's D-Luciferin reagent (cat# BLIV800A-1) and imaged on an IVIS Imaging System after 18 hours (lower left panel) and then later after 48 hours post DNA injection (lower right panel).



V. Licensing and Warranty

Limited Use License

Use of the BLIV Vectors (*i.e.*, the “Product”) is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.

The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.

This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

HIV Vector System

This product is for non-clinical research use only. Use of this Product to produce products for resale or for any diagnostic, therapeutic, clinical, veterinary, or food purpose is prohibited. In order to obtain a license to use this Product for these commercial purposes, contact the Office of Research and Technology Ventures at the Dana-Farber Cancer Institute, Inc. in Boston, Massachusetts, USA. This Product or the use of this Product is covered by U.S. Patents Nos. 5,665,577 and 5,981,276 (and foreign equivalents) owned by the Dana-Farber Cancer Institute, Inc.

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CMV Promoter

The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242

RFP and GFP Reporter Genes

This product contains a proprietary nucleic acid coding for a proprietary fluorescent protein(s) intended to be used for research purposes only. Any use of the proprietary nucleic acids other than for research use is strictly prohibited. USE IN ANY OTHER APPLICATION REQUIRES A LICENSE FROM EVROGEN. To obtain such a license, please contact Evrogen at license@evrogen.com.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

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Limited Warranty

SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a credit. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

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