

Bioluminescent and Fluorescent Imaging Vectors

Lentivectors and Minicircle DNAs for Non-invasive Monitoring of Cellular Dynamics *In Vivo*

Molecular imaging techniques to visualize cell kinetics in small animals have resulted in an explosion in the knowledge of tumors, infectious diseases, and stem cell biology. The sensitivity and accuracy of *in vitro* and *in vivo* cell monitoring 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). SBI has created BLI and FLI dual reporter lentivectors and minicircle DNA constructs and D-Luciferin reagents to perform molecular imaging *in vivo*.

Bioluminescent Imaging (BLI)

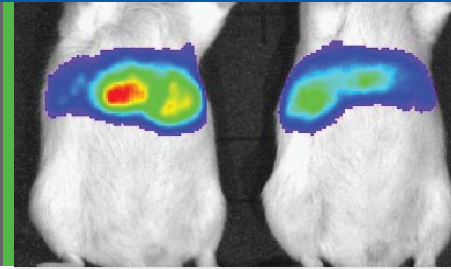
BLI uses light generated from a luciferase enzyme-substrate and an ultrasensitive cooled charge-coupled camera for signal detection. BLI may have the highest imaging sensitivity (even down to the one cell level), is high-throughput, easy-to-use system, and low cost.

Fluorescence Imaging (FLI)

FLI uses red/green fluorescent protein (RFP or GFP) as a signal. 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. FLI is typically coupled with BLI to provide an additional means for cell selection, sorting, thus gaining more histological and cellular positional information.

Positron Emission Tomography (PET)

PET is an enzyme-based positron emission tomography techniques that 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.



Highlights

- Four promoter choices
EF1, CMV, UbC and MSCV
- GFP + Luciferase
- RFP + Luciferase
- Lentivector plasmids
and packaged Virus
- Minicircle Parental
and pre-made Minicircles
- Bright D-Luciferin Reagent

Promoter choices

- EF1 alpha
- CMV
- UbC
- MSCV

Reporter combos

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

Vector options

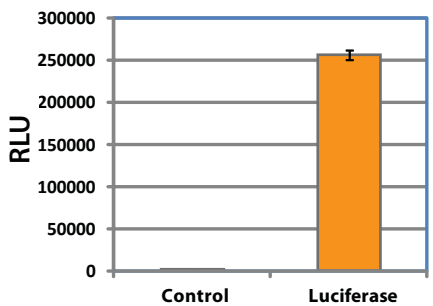
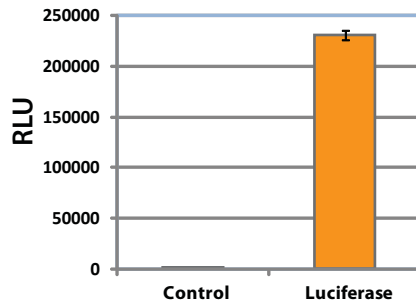
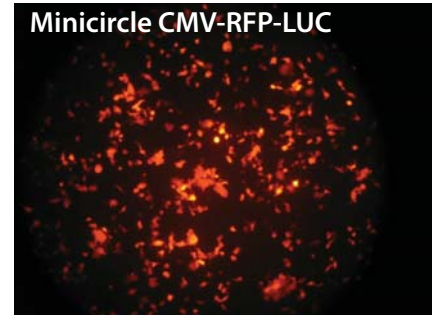
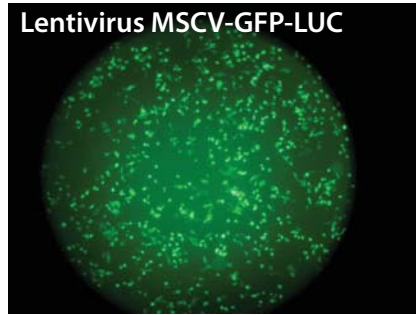
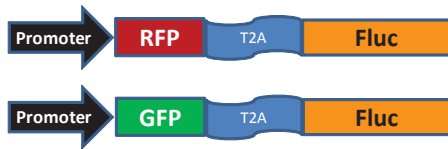
- Lentivector
- Minicircle DNA

Bioluminescent and Fluorescent Imaging Vectors

Create Dual Bioluminescent and Fluorescent Cell Lines

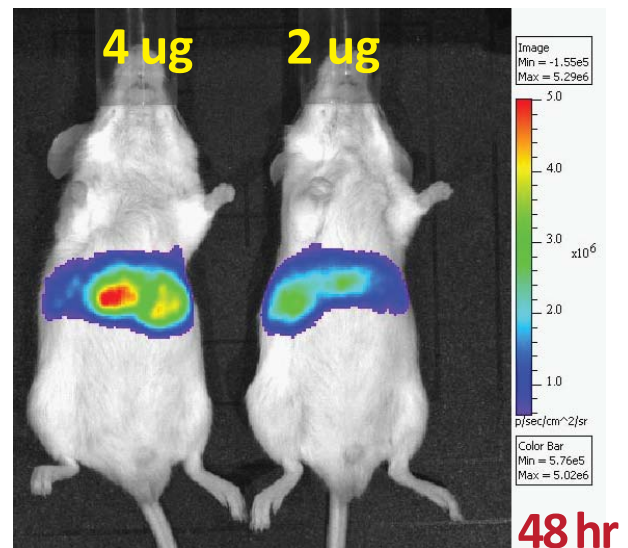
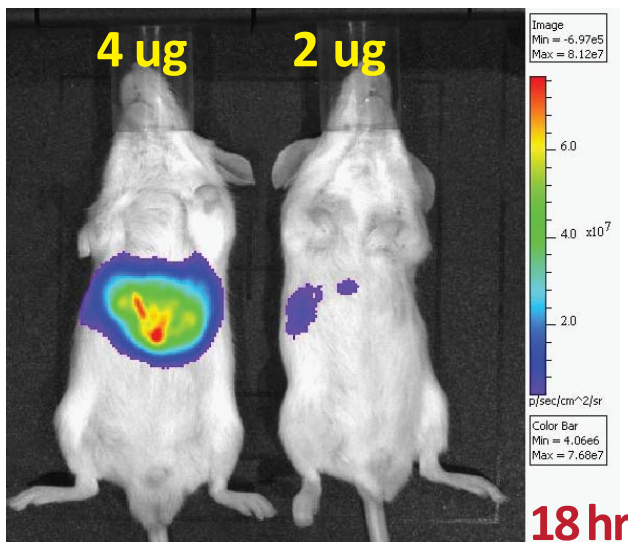
Use either BLIV lentiviral vectors to generate stable cell lines or utilize Minicircle vectors to make nonviral and long-lasting (several weeks) reporter cells.

- Promoter choices** **Reporter combos** **Vector options**
- EF1 alpha
 - CMV
 - UbC
 - MSCV
 - GFP-T2A-Luciferase
 - RFP-T2A-Luciferase
 - Lentivector
 - Minicircle DNA



Inject Bioluminescent and Fluorescent Cell Lines or Directly use BLI Vectors *In Vivo*

BLI 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 and imaged after 18 hours (lower left panel) and then later after 48 hours post-injection (lower right panel).



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System Biosciences, Inc.
265 North Whisman Rd.
Mountain View, CA 94043

Toll Free: 888.266.5066
Fax: 650-968-2277
Email: info@systembio.com
www.systembio.com

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