

**LentiGlo Product applications:** Lentiviruses expressing Gluc have applications in tumor imaging, studying survival of tumor cells and in monitoring survival and growth of implanted stem cells:

### **LentiGlo™ Lentiviral Vector System**

LentiGlo is a novel, highly efficient Lentiviral vector system in which the gene encoding Gaussia luciferase, expressed under control of the CMV promoter has been engineered into a proprietary optimized HIV-1 based Lentiviral vector that can be modified to accommodate additionally any desired gene, RNAi, promoter, and post-transcription or insulator element. Also, LentiGlo is free from the intellectual property (IP) constraints of other Lentiviral vector systems as this vector. There are two types of LentiGlo vectors, currently offered by Pluristem Innovations. The first are those that are engineered to express the gaussia luciferase alone or a firefly luciferase gene under control of the CMV promoter (Lenti-Gluc) or to express both a luciferase (under control of the CMV promoter) along with a GFP (e.g. Lenti-Gluc-GFP), that has been subcloned downstream of an IRES. **Gaussia luciferase is over a 1000 times brighter than the firefly and renilla luciferases. The red emitting luciferase from *Luciola Italica* is 75 times brighter than the red emitting luciferase from *Photinus pyralis* (Promega corp., USA) and the green emitting luciferase is approx 3 times as bright as the green emitting luciferase from the American firefly *Photinus pyralis***

### **The second category of vectors is the LentiGlo custom vectors:**

The GFP gene can be removed and replaced with an siRNA to inhibit a specific gene, or replaced with another gene of interest. And those that are designed to express a specific gene in target cells. Below you will find examples of a LentiMax vector that either silences or expresses a gene of interest. With LentiMax, you are in control of your vector design. You select the type of promoter to drive the expression of your sequence of interest

The **LentiGlo-Gluc** and the LentiGlo-Gluc-GP vectors are both priced at \$1500 and provide sufficient viral particles to transduce 500,000 to 1000,000 cells.

### **Background-Lentiviral Vectors**

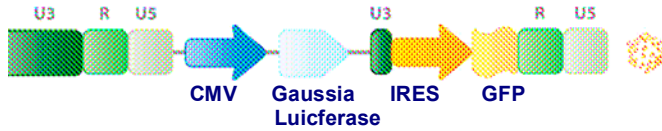
Lentiviral vectors (LVs) are viral-based gene delivery systems that can stably deliver genes or RNAi into primary cells or cell lines with up to 100% efficiency. LVs bind to target cells using an envelope protein which allows for release of the LV RNA containing the gene or gene silencing sequence into the cell. The LV's RNA is then converted into DNA using an enzyme called reverse transcriptase by a process called reverse transcription. The DNA pre-integration complex then enters the nucleus and integrates into the target cell's chromosomal DNA (see figure below).

Gene delivery is stable because the target gene or gene silencing sequence is integrated in the chromosome and is copied along with the DNA of the cell every time the cell divides. One of the discriminating features of LVs is their ability to integrate into non-dividing cells, in contrast to other vectors that either don't integrate efficiently into chromosomal DNA (e.g. non-viral, Adenoviral and Adenoviral-Associated vectors) or can only integrate upon cell division (e.g. conventional Retroviral vectors)

### **Advantages of using Gaussia luciferase (Gluc) as a reporter gene in the LentiGlo system**

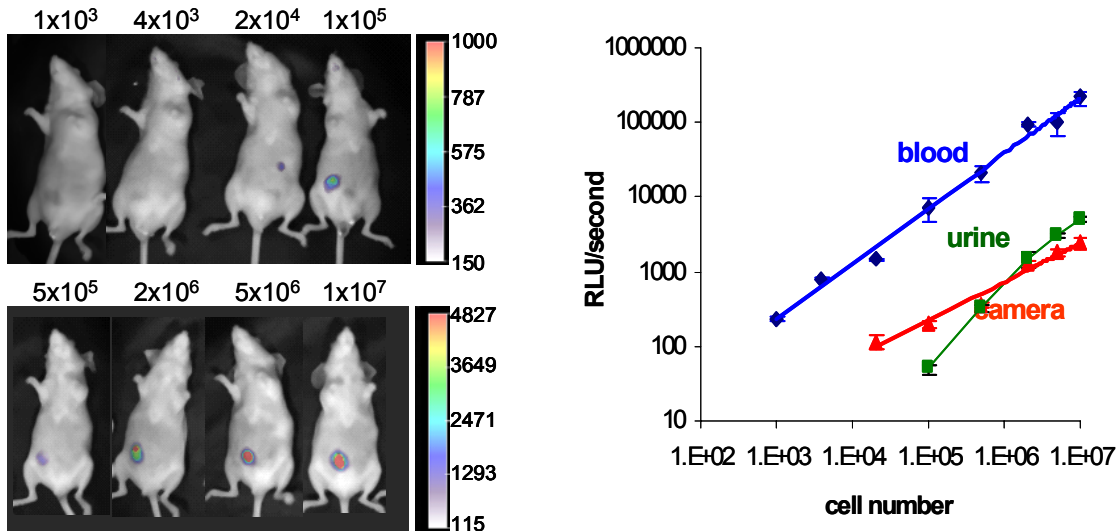
- Gluc is secreted; therefore activation can be monitored by assaying few microliter of conditioned medium with no need for cell lysis.
- Gluc is 2000-fold more sensitive than commonly used reporters such as luciferases from *Renilla* or firefly and the secreted alkaline phosphatase (SEAP).
- The GLuc assay reagent can detect as few as 10 mammalian cells expressing it .

## LentiGlo Lenti-Gluc-GFP expression vector



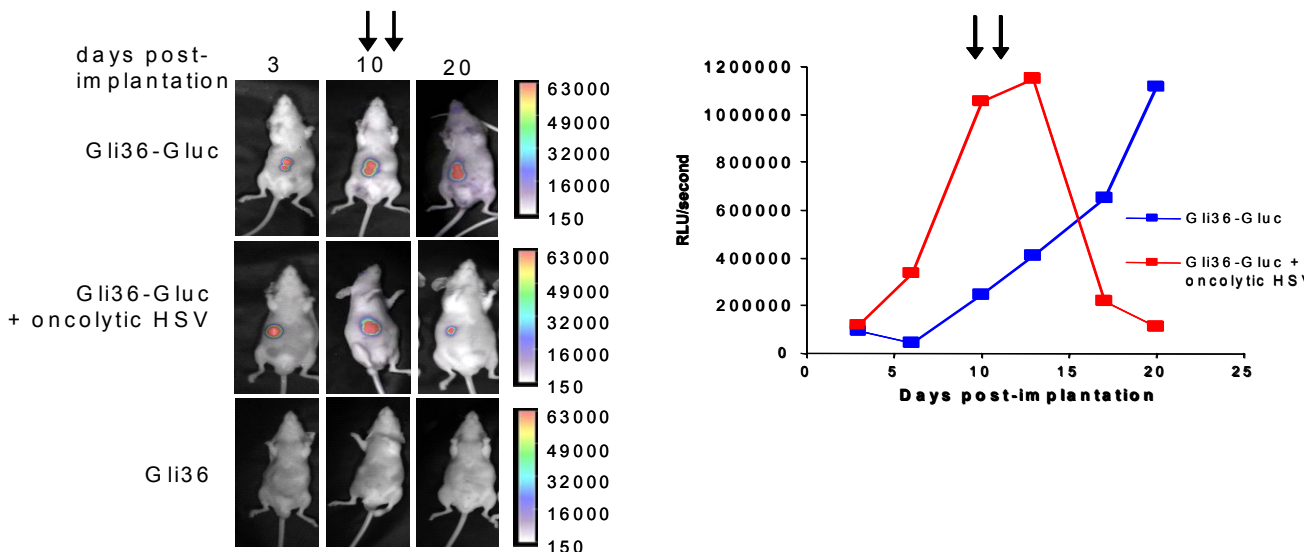
**Figure 1:** Gluc and GFP separated by an internal ribosomal entry site (IRES) were cloned in a self inactivating lentivirus vector. Lentiviral vectors expressing Gaussia luciferase, red and green emitting firefly luciferases and GFP are commercially available from Pluristem Innovations. Both luciferases are from the Italian firefly *Luciola Italica*.

### Gluc level in blood is linear with respect to implanted cell number



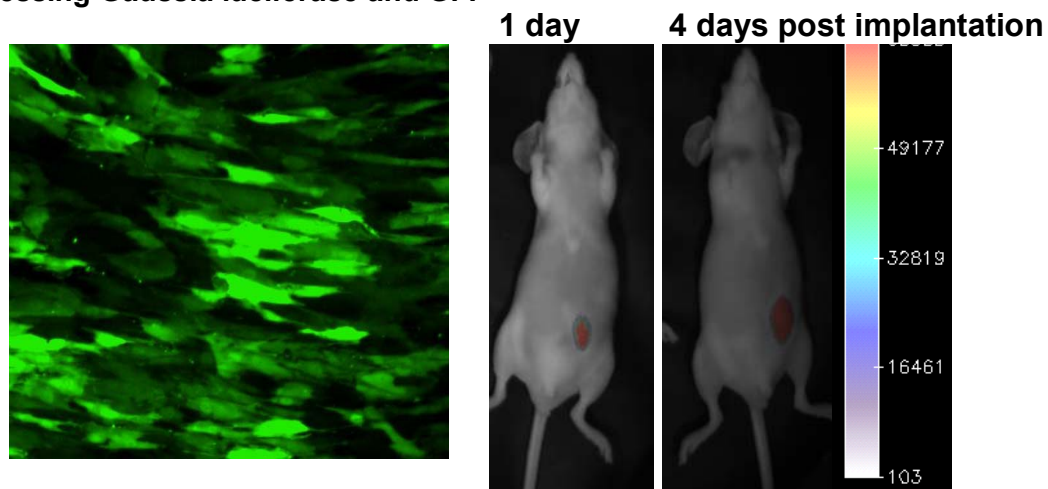
**Figure 2. (Left)** Different numbers of Gli36 human glioma cells expressing-Gluc (Gli36-Gluc) were implanted subcutaneously in mice and 3 days later, mice were injected i.v. with coelenterazine (4 mg/kg body weight) and imaged with CCD camera. **(right)** Total relative light units (RLU) per second were calculated for tumors in (red line). Gluc activity was measured in 5  $\mu$ L blood (blue line) or urine (green) after addition of 100  $\mu$ L 100  $\mu$ M coelenterazine and acquiring photon counts using a luminometer. **Data courtesy of Dr Bakhos Tannous, Massachusetts General Hospital, Harvard Medical School, Boston, MA)**

### Lentiviruses expressing Gluc have applications in tumor imaging, studying survival of tumor cells and in monitoring survival and growth of implanted stem cells:



**Figure 3.** Mice were implanted with one million Gli36-Gluc cells subcutaneously and tumor growth was monitored by both *in vivo* bioluminescence imaging (left) and the Gluc blood assay (right). At day 10 and 13 post-implantation, one set of mice was injected intra-tumorally (arrows) with an oncolytic HSV vector and another set with PBS (blue line). Gluc blood level from tumors treated with virus decreased showing that Gluc blood assay can be used to monitor cell death. (Data courtesy of Dr. Bakhos Tanoos, Massachusetts General Hospital, Harvard Medical School, Boston, MA)

**Efficient transduction and in vivo imaging of human mesenchymal stem cells with a lentivirus expressing Gaussia luciferase and GFP**

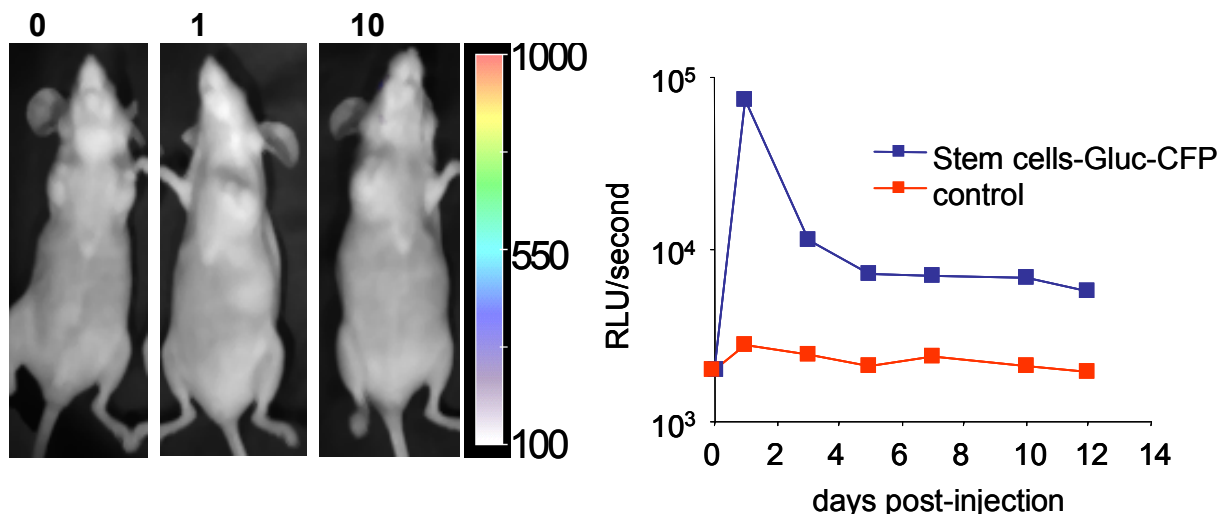


**Figure 4 :** Primary human bone marrow-derived mesenchymal stem cells were transduced with a lentivirus vector carrying the expression cassette of Gaussia luciferase and GFP, separated by an IRES element, under control of the CMV promoter (VMV-Gluc-IRES-CFP) at an MOI of 30. The results indicate that the transduction efficiency was nearly 100% (left). One million of these cells were mixed with matrigel and implanted subcutaneously in nude mice. At different time points, mice were injected with coelenterazine and imaged using a CCD camera (right). The signal increased over time showing that these cells proliferated *in vivo*.

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**Gluc blood assay to monitor circulating stem cells:**

**Days post-injection:**



**Figure 5.** One millions stem cells expressing Gluc and GFP or PBS control were injected i.v. in nude mice. Prior to injection and at several time-points the Gluc activity was monitored using the CCD camera and in 20  $\mu$ L blood samples using the luminometer. At no time point the CCD camera was able to detect the stem cells, however, the Gluc level in blood indicated that a significant number of cells survived the injection and did not proliferate (Data courtesy of Dr Bakhos Tannous, Massachusetts General Hospital, Harvard Medical School, Boston, MA)

**REFERENCES CITING USE OF LENTIVIRAL VECTORS EXPRESSING Gluc :**

1. BA Tannous, DE Kim, JL Fernandez, R Weissleder, and XO Breakefield. (2005) Codon-optimized Gaussia luciferase cDNA for mammalian gene expression in culture and in vivo. *Mol Ther*, Mar 2005; 11(3): 435-43.
2. Wurdinger T, Badr C, Weissleder R, Breakefield X and Tannous B. Gaussia luciferase for ex vivo monitoring of in vivo processes. *Nature Methods* (in press)

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**ORDERING INFORMATION: (Discounts available for bulk purchase)**

	<b>LentiPower- Positive control luciferase expression vectors withCMV promtoer</b>		
#LP-04	LentiPower-pCMV-Gluc Expression vector expressing Gaussia luciferase under control of the CMV promoter, Lentivirus packaging mix, Targefect-F-2, GAR-1 reagent (1000 reahn)	10 reactions	\$1400.00
#LP-05	LentiPower-pCMV-RedFluc Expression vector expressing red firefly luciferase under control of CMV promoter, Lentivirus packaging mix, Targefect-F-2, FLAR-1000 (1000 assays)	10 reactions	\$1400.00
#LP-06	LentiPower-pCMV-GreenFluc Expression vector expressing green firefly luciferase under control of CMV promoter, Lentivirus packaging mix, Targefect-F-2, FLAR-1000 (1000 assays)	10 reactions	\$1400.00
#LP-07	Lenti-Gluc-GFP Expression vector expressing Gaussia luciferase under control of the CMV promoter followed by GFP expressed under an IRES, Lentivirus packaging mix, Luciferase assay reagent GAR-1 reagent (1000 reahn)	Ready to use lentivirus	\$2000.00
LentiGlo-hMSC	Human adult mesenchymal stem cells (adipose tissue-derived) transduced with a lentivirus co-expressing Gluc (Gaussia luciferaseexpressed under control of the CMV promoter) and GFP	2 million cells  (cryopreserved)	\$2500.00

LentiCustom Service	Custom lentiviral vector expressing gene of choice under control of CMV promoter (available with choice to co-express a second reporter gene)	Available in plasmid format, ready to use lentivirus or stem cells transduced with custom lentivirus	Enquire for pricing
	<b>Transfection reagents for transient gene transfer into mesenchymal stem cells</b>		
PLU-T1	Plurifect with enhancer	100 reactions	\$250.00
#0060	Plurisilence transfection reagent for siRNA delivery into stem cells	100 reactions	\$200.00
PLU-P	Pluriport protein delivery kit for efficient intracellular delivery of functionally active proteins/peptides into stem cells (tested with human mesenchymal stem cells from bone marrow or adipose tissue and with bone marrow-derived hematopoietic stem cells, Includes a reporter protein to easily assess efficiency of protein delivery)	50 reactions	\$300.00
	Cryopreserved human adult mesenchymal stem cells (adipose tissue-derived)- 3 million cryopreserved cells	3 million cryopreserved cells	\$400.00
hMSC-01			
hMSC-BM	Basal medium for maintenance of stem cells	500 ml	\$130.00
hMSC-GM	Growth medium which promotes rapid growth of human mesenchymal stem cells (adipose tissue derived)	500 ml	\$150.00