

LentiGlo™ Catalog # LP-42 -A prepackaged lentivirus co-expressing secreted Gaussia luciferase and intracellular bright red-emitting firefly luciferase (also available as lentiplasmid, (**Catalog # pLP-42**) for improved bioluminescent imaging and assessment of *In vivo* gene transfer

Tracking the fate and function of cells or genes *In vivo* is paramount for the development of rational cell-based and gene-based therapies. Sensitive imaging in whole organisms to visualize localization of the bioluminescent signal requires emission of red to near IR (>600 nm) light, as these wavelengths are significantly less absorbed by tissue. Survival of transplanted cells on the other hand is best quantitated by measurement of an ultrasensitive secreted luciferase in the blood. Lentiviral vectors constitutively co-expressing divergent luciferases which use different substrates enable monitoring both cellular localization as well quantitative assessment of survival of implanted cells. The dual luciferase-expressing lentivirus (Lenti-UBC-RedFLuc-2A-GLuc) co-expresses a bright red-emitting firefly luciferase (RedFLuc) with emission max 617 nm and secreted Gaussia luciferase (GLuc). The RedFLuc enables visualization of bioluminescent cells in deep seated tissue. The GLuc reporter is 1000 times brighter than Firefly luciferase reporters and allows measurement of cell survival using a simple rapid assay (Fig. 2B)

TABLE 1: Bioluminescence of tumor cell lines transduced with a lentivirus co-expressing secreted Gaussia luciferase and a Red-emitting firefly luciferase under control of the CMV promoter (Lenti-CMV-GLuc-T2A-RedFLuc-T2A-Puro, Catalog #LP-42, Targeting Systems, CA).

CELL LINE	PHOTONS/SEC/CELL Red-emitting Firefly Luciferase	PHOTONS/SEC/CELL Secreted Gaussia luciferase
MDA MB231	5000	100,000
CT26WT	2000	50,000
ST941C	5000	70,000
BT474	2000	40,000

ADVANTAGES:

Robust expression of both luciferases and proven performance of both luciferase reporters (Table1, Figures 2-4) for improved bioluminescent imaging (RedFLuc enables visualization of deep tissue GLuc enables quantitative measurement of cell survival *In vivo*)
Puromycin resistance for easy selection

Figure 1: MDA MB231, CT26WT, BT474, ST941C cells in culture were grown in 24-well dishes (50,000 cells/well) and transduced with Lenti-CMV-GLuc-T2A-redFluc-T2A-Puro (catalog no LP-42 , Targeting Systems, CA) as per the lentivirus transduction protocol at the following link at an Moi of 10. The cells were incubated at 37⁰ C for 2 days and photon flux was calculated by imaging with D-luciferin (for RedFLuc expression) and native coelenterazine (for GLuc expression). The bioluminescent cell lines were made by Dr Aleksey Yevtodiynko at Swiss Lumix, Lausanne Switzerland.

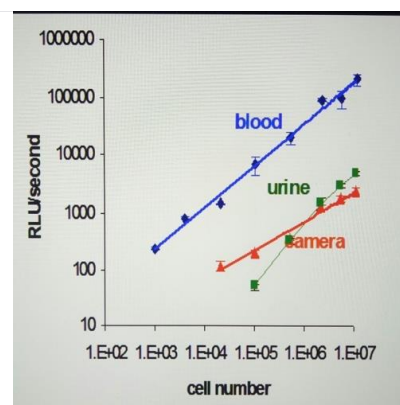
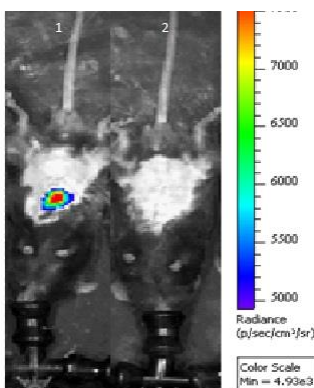


Figure 2A: Intrahepatic luciferase luminescence detected by real-time imaging (Xenogen IVIS® Spectrum Bioluminescence). (1) Strong luminescence signals are noted on the right upper abdomen of a mouse treated with magnet 14 days post intrahepatic injection of mEP cells labeled with UBC-RedFluc-tdtomato. (2) No signal seen in negative control. **Data courtesy of Dr Jeffrey Fair's lab, Cedar Sinai Hospital, Los Angeles, CA.**

Fig 2B Human glioma cells (GLi136-gLuc) were implanted subcutaneously in mice and 3 days later mice were injected iv with coelenterazine. Total RLU per second was calculated for tumors (red line). GLuc activity was also measured in blood (blue) or urine (green) after addition of coelenterazine and measurement in a luminometer. (Data courtesy of Dr Bakhos Tannous, Massachusetts General Hospital, MA)

Efficient reporter for assessing efficacy of gene transfer

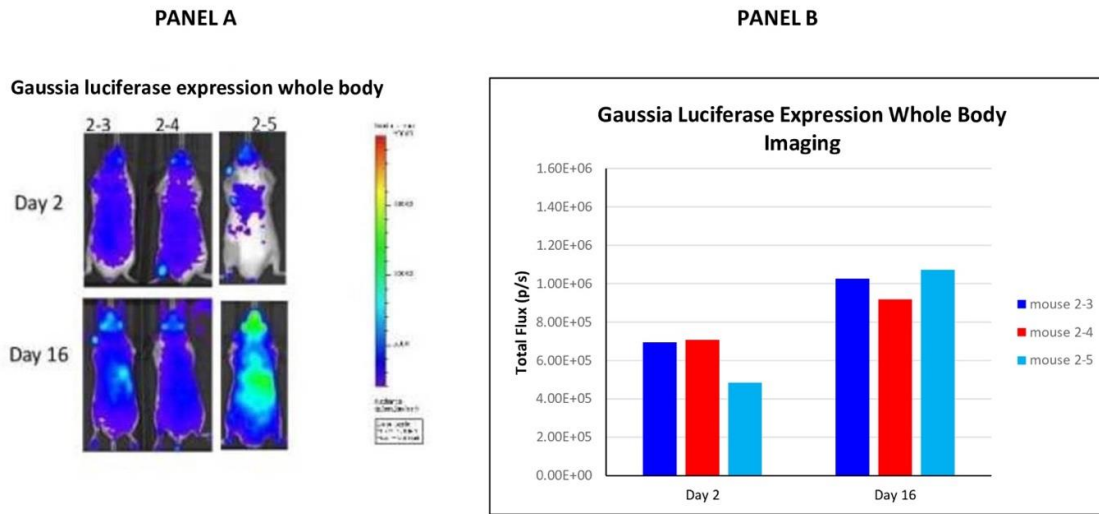


Figure 3: Bioluminescent imaging of secreted Gaussia luciferase expression in transduced mice by injecting coelenterazine at day 2 and 16 after intramuscular injection of 20 ug pLenti-UBC-GLuc-T2A-redFLuc lentiplasmid DNA (in 20 ul water) complexed with 10 ul of InVivo-Targefect, incubated atRT for 30 mins and then injected intra-muscularly into the mouse calf muscle. The expression vector used in these studies was a lentiplasmid co-expressing two different luciferase reporters. Intracellular red-emitting firefly luciferase and secreted Gaussia luciferase. Panel A: Bioluminescent imaging of GLuc expression (whole body imaging) at days 2 and 16 following intramuscular gene transfer Panel B: Photon Flux for BLI results of mice shown in Panel A (bioluminescent imaging of GLuc expression (whole body imaging) at days 2 and 16 following intramuscular gene transfer)

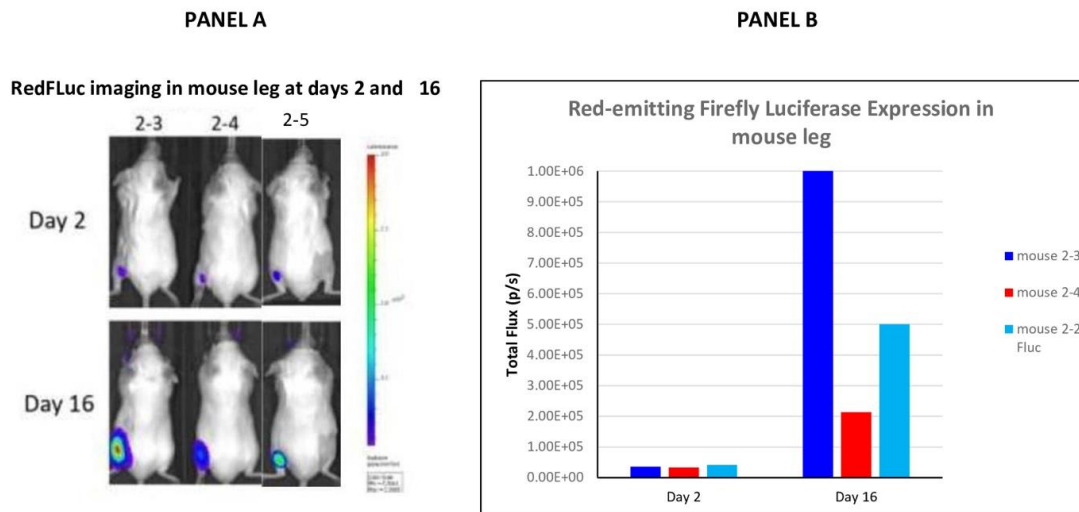
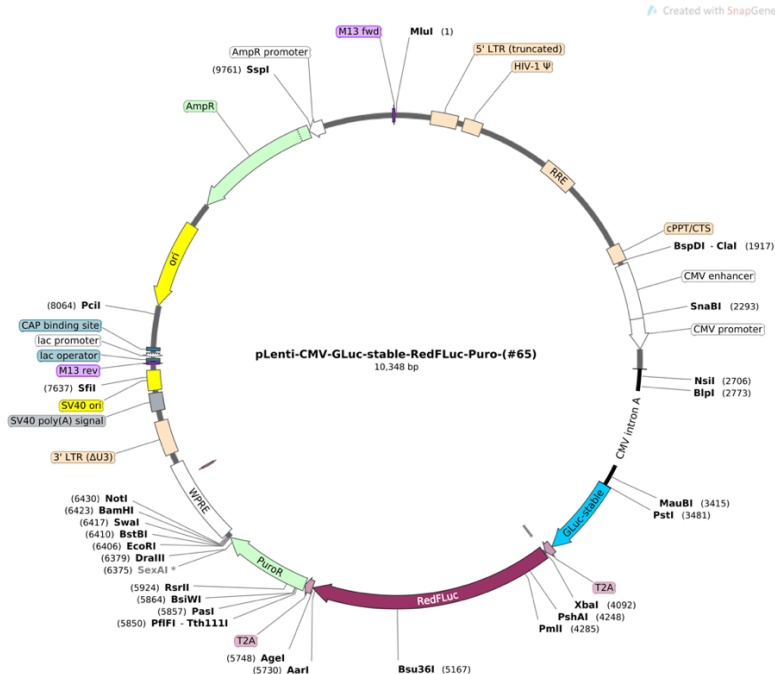


Figure 7: Bioluminescent imaging of Firefly luciferase expression in transduced mice by injecting luciferin at day 2 and 16 after intramuscular injection: 20 ug pLenti-UBC-GLuc-T2A-redFLuc lentiplasmid DNA (in 20 ul water) complexed with 10 ul of InVivo-Targefect, incubated atRT for 30 mins and then injected intra-muscularly into the mouse calf muscle. The expression vector used in these studies was a lentiplasmid co-expressing two different luciferase reporters. Intracellular red-emitting firefly luciferase and secreted Gaussia luciferase. Panel A: Bioluminescent imaging of FLuc expression (imaging of mouse leg) at days 2 and 16 following intramuscular gene transfer . Panel B: Photon Flux for BLI results of mice shown in Panel A (bioluminescent imaging of FLuc expression (imaging of mouse leg) at days 2 and 16 following intramuscular gene transfer)

ALSO AVAILABLE AS A LENTIPLASMID (CATALOG # pLP-42) SO YOU CAN PACAKGE LENTIVIRUS YOURSELF



ORDERING INFO:

Catalog #	Product name	Description	Price
LP-42	LentiGlo™ Lenti-CV-GLuc-T2A-RedFLuc -T2A-PURO	Lentivirus co-expressing secreted gaussian luciferase and intracellular Red-emitting firefly luciferase (400ul high titer lentivirus) , pLP-42 lentiplasmid, 1000 assays of firefly luciferase assay reagent (FLAR-1)	\$2000
pLP-42	pLenti-CMV-GLuc-T2A-RedFLuc	Lentiplasmid co-expressing	\$750
LP-42L	LentiGlo™ Lenti-CV-GLuc-T2A-RedFLuc -T2A-PURO	LentiGlo™ Lenti-CV-GLuc-T2A-RedFLuc -T2A-PURO ready to use lentivirus (200ul high titer lentivirus (virus only)	\$1000

To place orders, please call us at 1(888)818-2446 or (619)562 1518 or FAX us at (619)562 1326. You can also contact us via email info@targetingsystems.net

Targeting Systems
 1453 N Cuyamaca St
 El Cajon, CA 92020
 Telephone: 888-818-2446
 Fax: 619-562-1326
www.targetingsystems.net