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Introducing LentiGlo™

Ready-to-use lentiviruses expressing three novel luciferases, the brightest and the best

- Gaussia luciferase
- Red-emitting Firefly luciferase from Luciola Italica
- Green-emitting, secreted, Renilla luciferase

Advantages:

The lentiGlo products offer a unique advantage of efficiently and stably transfecting even the most difficult cell types including primary cells including non-dividing cells, neuronal cells and stem cells. Since three different ultrasensitvite luciferase reporters are offered, it is now possible to study promoter activities of three different promoters in the same group of transfected cells or to study expression of the same gene in three different cell types simultaneously (eg in co-culture). Their use circumvents the use of transfection reagents, saves considerable time and allows for the generation of stably transfected primary cells in a very short time due to integration of the lentivirus into the host cell. The LentiGlo vectors based on advanced lentiviral technology are SIN vectors (self inactivating) and therefore safe for use. . 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). The sensitivity of the luciferase reporters in the LetiGlow system is sufficient for studying reporter gene expression in a single infected cell.

Features of the LentiGlo luciferase reporters:

Gaussia luciferase is the brightest, known secreted luciferase

The red-emitting modified Luciola luciferase we offer is over 1000 times brighter than native Luciola Italica luciferase. The secreted green-emitting Renilla luciferase gives the brightest intracellular signal and being red-shifted allows for better tissue penetration. It has been engineered for improved stability in vivo.

Applications:

Now there is enough light to do things hard to do before

- Tumor Imaging. Red-emitting luciferase permits imaging of deep-seated tissues
- Studying growth and survival of implanted cells
- More sensitive reporters for HTS
- Tracking implanted stem cells.
- Live cell assays using chemiluminescent or fluorescent reporters
- Measurements of promoter activity and gene expression profiles without killing the cells

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LentiGlo™ Lentiviral Vector System

LentiGlo is a novel, highly efficient Lentiviral vector system in which the gene encoding the 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, including RNAi,. promoter, and post-transcription or insulator element LentiGlo vectors, currently offered by Targeting Systems are either engineered to express the luciferase gene alone under control of the CMV promoter(Lenti-Gluc), or along with a GFP (green fluorescent protein) or an RFP (red fluorescent protein). Gaussia luciferase is over a 1000 times brighter than the firefly and Renilla luciferases. The red- emitting luciferase from Luciola Italica is 1000 times brighter than the native red-emitting Luciola luciferase The green-emitting secreted Renilla luciferase mutant is about 30 times brighter than native (human codon optimized) Renilla luciferase

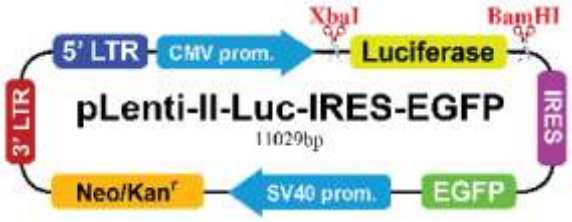
LentiGlo custom vectors: In these vectors the GFP gene can be removed and replaced with a second gene or with an siRNA to inhibit a specific gene of interest. Thus the Lenti-custom vectors encode sequences that either silences or expresses a gene of interest. With our alternate product line, LentiPower, the user has the ability to replace the CMV promoter with the promoter of interest. The LentiGlo products provide sufficient viral particles to transduce 10 million cells.

Gene delivery using LentiGlo 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 secreted luciferase. Gaussia luciferase or Renilla luciferase as a reporter gene in the LentiGlo system:

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

Plasmid Map of the pLentiCMV-GLuc-IRES-EGFP and pLentiCMV-RedFluc-IRES-GFP vector:



Please note that this vector has neomycin as a marker for making stable cell lines expressing Gaussia lcuiferase. Or red Firefly luciferase. Alternately the GFP can be used to identify and separate transfected cells using FACS.

An alternate version of the LentiGlo vector encoding Gaussia luciferase alone under control of the CMV promoter but no GFP is also available.

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The maps of the pLentiCMV-RedFluc plamsid is similar to the one above wherein the Gaussi alcuiferase is replaced by the red'emitting firefly lcuiferase Sequences (human codon optimized) encoding Gaussia luciferase and Red'emitting firefly luciferase genes are given below. We are unable to disclose the complete squence of our Lenti plasmids due to licensing issues.

Sequence of Gaussia luciferase

Atgggagtcaaagttctgtttgccctgatctgcatcgctgtggccgaggccaagcccaccgagaacaacgaagacttcaacatcgtgg
ccgtggccagcaacttcgcgaccacggatctcgatgctgaccgcgggaagttgcccggcaagaagctgccgctggaggtgctcaaaga
gatggaagccaatgcccggaaagctggctgcaccaggggctgtctgatctgcctgtcccacatcaagtgcacgcccaagaTgaagaag
ttcatcccaggacgctgccacacctacgaaggcgacaaagagtccgcacagggcggcataggcgaggcgatcgtcgacattcctgaga
ttcctgggttcaaggacttggagcccatggagcagttcatcgcacaggtcgatctgtgtgtggactgcacaactggctgcctcaaagg
gcttgccaacgtgcagtgttctgacctgctcaagaagtggctgccgcaacgctgtgcgacctttgccagcaaGatccagggccaggtg
gacaagatcaaggggccggtggtgactaagcggccgctcgagcatgcatctag

Sequence of Red-emitting firefly luciferase from Luciola Italica

ATGGAAAcaGAAAGAGAAGAAaAcgTTgtcTAcGGcccacTGccATtctaccCGATcGAGGAGGgCTcTGccGGCAtcCAATtGCaca
aGTaCATGcAACAaTACGccAAgCTcggcGCcaTCgcCTtCAgTAACgcCCtGACAGgCGtCGaCAtCAGCtaCCAGcAGtacttcga
catcacgtgCAgaCTcgcCGaggcTAtgaagaacTAcggcatgaagcCAgaaGGaCAcATcGCtctCTgTAGCGAGAaCTgCGAAGAG
ttcTTCAttcCTgTTcTGgCTGGtctTTaCAtCGGAGTtaCAGtCGcgcCAACtaaCGAaAttTATAcACtTAgaGAGCtGAaCCaCA
gtcTGggGATAgccCAaccTAcTAtcgtATtcTCtagcAGGAAGGGcCTgccCAAAgTGcTTGAGGTGcAGAAGAcCGTGacTTgCAt
CAAaAccatTGtcATcctgGACAgTAAGgtcaacttcggcggttatgaCTgcgtagagaccTTcattaagaaacaCGtCGagctgggc
ttTCctGCcaCCtcATtTGtgccCAtcGAcgtCAAAGaCcgGAAGCAccACaTTgCTctGCttatgaactcttccggttcCAcagggc
tgcccaaaggagtagagatCActCAcgaggcccTGgtCACGagATtctCTcacgcTAAGGAccCTATaTAcggcAAtCAGGtgGCccc
AGGTaccgctatcctgactgtcgtgcctttccaccacggcttcggAAtgttcactactttgggctactttgcctgcggttaccggatt
gtcatgcttactaagttcgACgaggagcttttcctGCgcacacttcaggattacaagtgcactacagtaatcctggtgccgacactgt
tCGcaattctTAATAGgTCTGAGctccTTGATAAGtTTGAcctCTctAAccTGaCTGAAATAgcCAGCGGtGGTGctccacTTgcCAA
GGAGATcGGcgAGgcTGtTGcAAGAAgAttCAACctccCAggcgtccggcagggatatggactcaccgagactaccagtgcctttatc
atcactcctaagggcgacgaCAagcCGggagccagcggCAaggtCGtgcCTctgttCAaggtgaagattattgacctCGatacCAaga
aaacgttgggTGtcaacagacggggagAAatcTGcgtgaAAggACcatctcttatgttgggatacacgaacaatcctgaagccaccag
agaaactattgacgagGAaggCTGGcTGcaCACGGGTGaCAtCGGGTAcTACgaCGAgGaTGAGcACttCTtTATAgtcgaccgcctg
aaatctctcattaagtataaaggaTAccaagtgcCAccagctgaaCTggagtctgtgctcctgcaacaccctaacattagagatgctg
GTgtggccgGGGTtcccGaCagcgaggcaggcgagctgcctggagcCGtcgttgTGaTGgaaaagggaaagaCAaTGactgagaaaga
aatcgtagactatgtaaactccCAggtggtcaaccacaagcggCTgaggggcGgcgtgcggttcgtagatgaagtccccaaggggctc
acaggaaagatcgacgcgaaagttatcagggagatactcaagaaacctcaagcaggtgggtag

Note:

LentiGlo vectors expressing secreted green-emitting Renilla luciferase have different maps. The Green Renilla mutant luciferase is expressed under control of the CMV promoter either alone pLentiCMV-GrRenLuc plasmid or co'expressed with a fluroescnt protein (Turbo Red or Katushka) under control of the phosphoglycerokinase promoter. The lenti plasmids expressing Renilla luciferase have Puromycin resistance.

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All LentiGlo vectors show excellent performance. The data below uses Gaussia lcuifersae as an example

Data illustrating applicability of LentiGlo vectors for studying cell growth and survival in vivo:

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

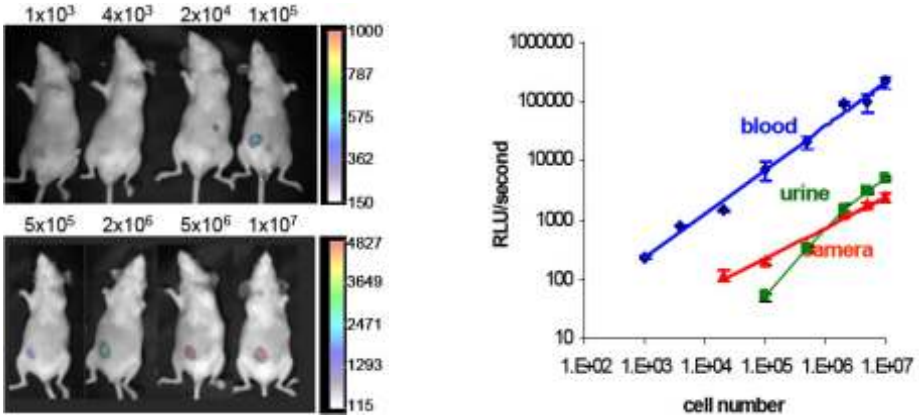

Figure 2: (Left) Different numbers of Gli36 human glioma cells expressing-Gluc (Gli36-Gluc) were implanted subcutaneously in mice and 3 days later, mice were injected i.v. with coelenterazine (4 mg/kg body weight) and imaged with CCD camera. (right) Total relative light units (RLU) per second were calculated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

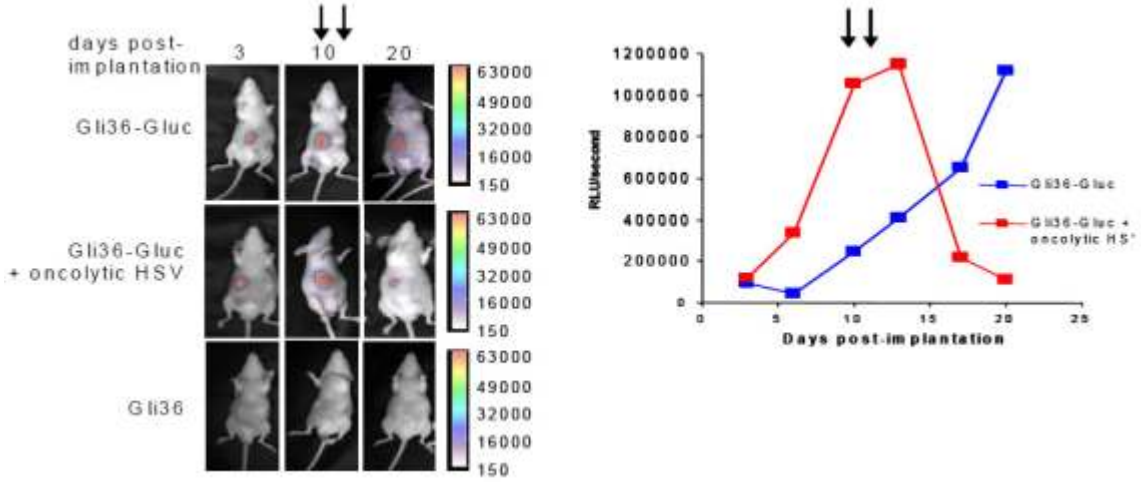

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Figure 3: Mice were implanted with one million Gli36-Gluc cells subcutaneously and tumor growth was monitored by both in vivo bioluminescence imaging (left) and the Gluc blood assay (right). At day 10 and 13 post-implantation, one set of mice was injected 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 Tanoous, Massachussetts 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.

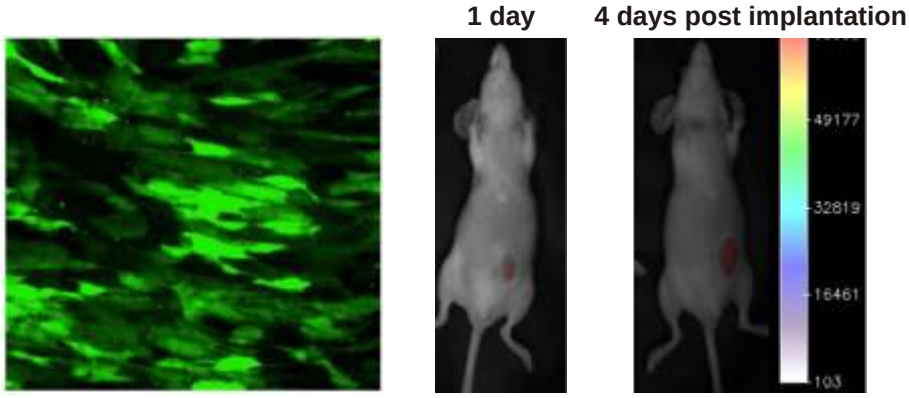

Figure 4: Primary human bone marrow-derived mesenchymal stem cells were transduced with a lentivirus vector carrying the expression cassette of Gaussia luciferase and GFP, separated by an IRES element, under control of the CMV promoter (VMV-Gluc-IRES-CFP) at an MOI of 30. The results indicate that the 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.

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

Gluc blood assay to monitor circulating stem cells:

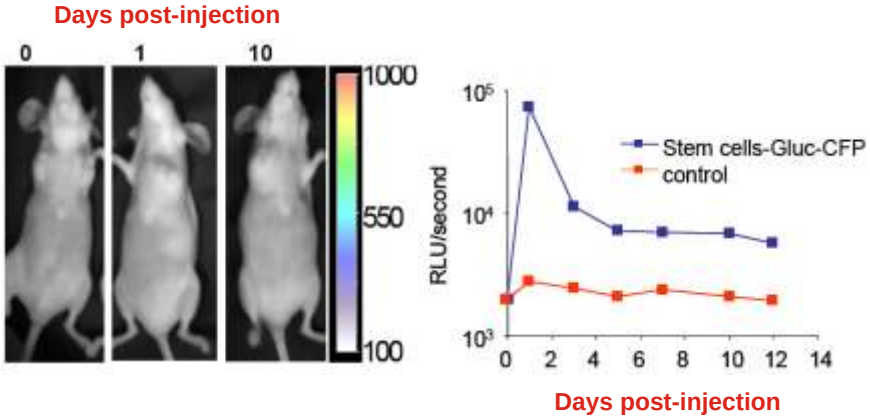

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Figure 5: One millions stem cells expressing Gluc and GFP or PBS control were injected i.v. in nude mice. Prior to injection and at several time-points the Gluc activity was monitored using the CCD camera and in 20 µL blood samples using the luminometer. 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, Massachussetts 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

Ordering Information

Note: Products are sold for research purposes only. Commercial use-users should obtain an appropriate License. Note: For licensing of Gaussia luciferase based products please contact Li Bryan at Prolume 928-367-1200

Catalog No.	Name of LentiGlo plasmid	Description	Price
LP-07	PLenti-CMV-GLuc-IRES-GFP	Lentivirus expressing Gaussia luciferase and GFP, pLenti-CMV-Gluc-IRES-EGFP plasmid, 1000 assays of the Gaussia luciferase assay reagent GAR-1.	$2000
LP-08	Lenti-CMV-RedFluc-IRES-EGFP	Lentivirus expressing Red firefly luciferase and GFP, pLenti-CMV-RedFluc-IRES-EGFP plasmid, 1000 assays of the firefly luciferase assay reagent FLAR-1.	$2000
LP-09	pLenti-CMV-GrRenLuc	Lentivirus expressing Green secreted Renilla , pLenti-CMV-GrRenLuc plasmid, 1000 assays of the luciferase assay reagent RLAR-1.	$2000
LP-10	pLenti-CMV-GrRenLuc-RFP	Lentivirus expressing Green Renilla luciferase under control of CMV promoter and RFP under phosphoglycerokinase promoter, pLenti-CMV-GrRenLuc-IRES-0RFP plasmid, 1000 assays of the luciferase assay reagent RLAR-1.	$2000
Lenti-Custom	Lenti-Custom	Any of the above expressing luciferase under your promoter of interest (subject to availability)	$2500.00

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LentiGlo protocol

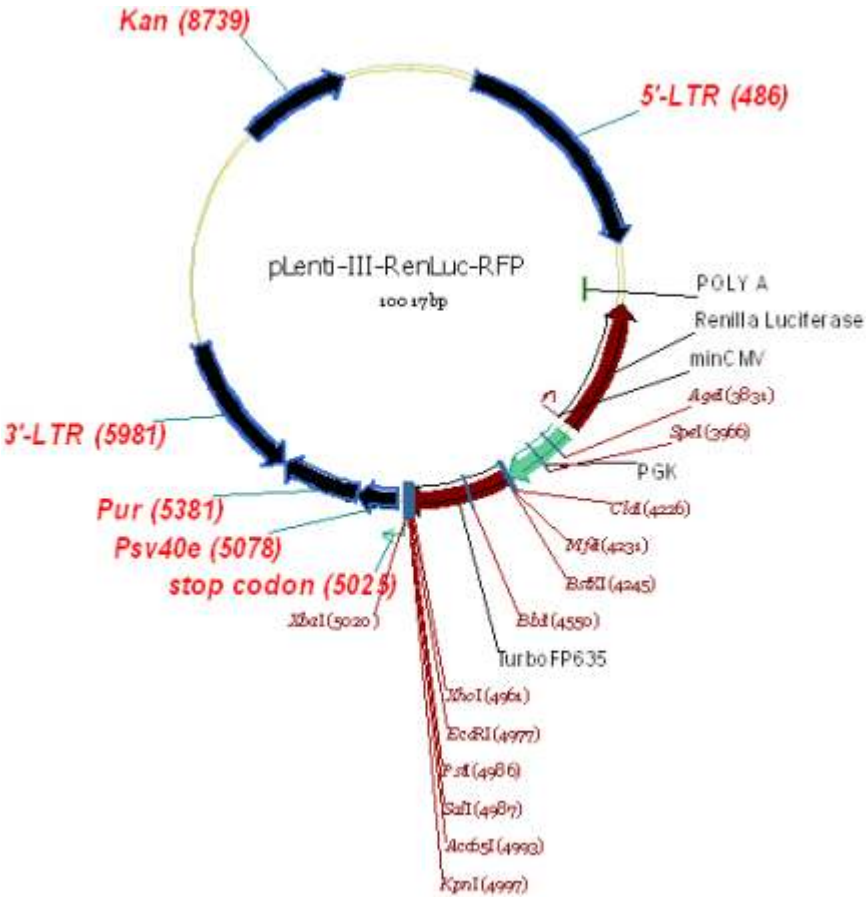
We normally transduce cells at MOI 10-50 depending on cell types. most human tumor cells infect really well at MOI10. Polybrene does increase transduction efficiency, but some cells such as primary cells do not like it. you can obtain it from Sigma Chemicals, MO, USA. We use it at a concentration of 10ug/ml (in PBS)

-Plate cells in 6-well plate at 70-80% confluency on day 1
-On day 2, remove media and add 1 ml of media containing lentivirus (MOI 10-50 use 20ul, 100 ul of LentiGlo ready to use lentivirus provided) and 10 ug/ml polybrene (optional and not recommended for primary cells)
-On day 3 aspirate the supernatant media, wash cells and re-place with fresh media.
Transfer cells to bigger plate once they are confluent.

Assay for luciferase acitivty in the supernatant or lysate using the luciferase assay protocol provided with the LentiGlo product

Please call tech support 1-866-620-4018 if you need more information or need clarification on the protocol. A more detailed protocol will be provided with the product

Plasmid Map of the plenti-CMV-GrRenLuc-RFP vector:



Also available as Lenti vector expressing Green –emitting renilla luciferase alone without EGFP.

Please note that Turbo Red (Katushka) is a proprietary gene from Evrogen, Russia and commercial use of the same requires a license from Evrogen.

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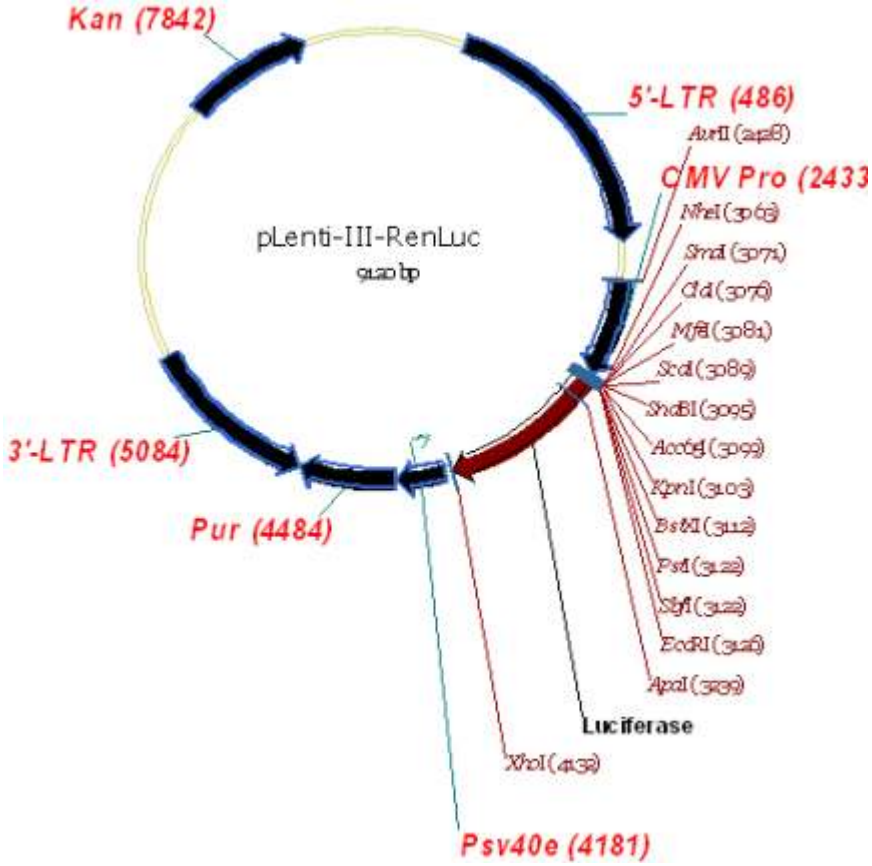
Plasmid Map of the plenty-CMV-GrRenLuc plasmid:



Sequence of secreted Green Renilla luciferase mutant gene is given below

Signal peptide (MLLKVVFAIGCIVVQA) is a synthetic codon-optimized cDNA sequence (for mammalian cell expression), encoding a 330-aa polypeptides .

ATGTTGTTGAAAGTTGTGTTTGCTATTGGATGTATCGTAGTGCAGGCTATGGCCTCAAAAGTGTACGATCCGGAGCAGCGGAAGAGGA
TGATCACGGGGCCCCAATGGTGGGCACGATGCAAGCAGATGAATGTGTTGGACAGTTTCATTAACTACTACGACAGCGAGAAACACGC
GGAGAACGCAGTGATATTCCTGCACGGCAATGCAACCAGTAGCTATCTGTGGAGACACGTGGTGCCTCATATTGAGCCGGTCGCTAGA
TGCATTATTCCCGATCTTATTGGAATGGGGAAATCCGGAAAGAGTGGAAATGGATCATATAGGCTCCTCGATCATTATAAATATCTGA
CTGCTTGGTTTGAATTGCTCAATCTGCCCAAGAAAATCATCTTTGTAGGACATGATTGGGGCTCCGCCCTTGCTTTTCATTATGCCTA
TGAACACCAGGATCGGATCAAGGCTATTGTTCACATGGAGAGCGTGGTGGATGTGATTGAATCATGGATGGGTTGGCCGGATATAGAA
GAAGAGCTGGCGCTGATTAAATCTGAGGAGGGCGAGAAGATGGTACTCGAAAATAACTTCTTTGTCGAGACGGTACTGCCCAGTAAGA
TCATGCGCAAACTGGAGCCTGAAGAGTTTGCGGCTTACCTGGAACCCTTCAAGGAGAAGGGAGAGGTGAGGAGACCGACCCTGTCATG
GCCTCGGGAAATTCCGCTGGTCAAAGGAGGGAAGCCAGACGTCGTCGCCATTGTCCGGAATTACAACGCTTACCTCCGCGCTAGTGAC
GACCTGCCTAAACTCTTCATCGAATCAGATCCTGGTTTCTTTAGTAACGCCATCGTCGAGGGCGCCAAGAAGTTTCCAAACACCGAAT
TTGTTAAAGTCAAAGGACTTCACTTCCTCCAGGAGGATGCGCCCGATGAAATGGGAAAGTATATCAAATCCTTCGTGGAGAGGGTCTT
GAAGAATGAGCAGAGGTCCATCtag

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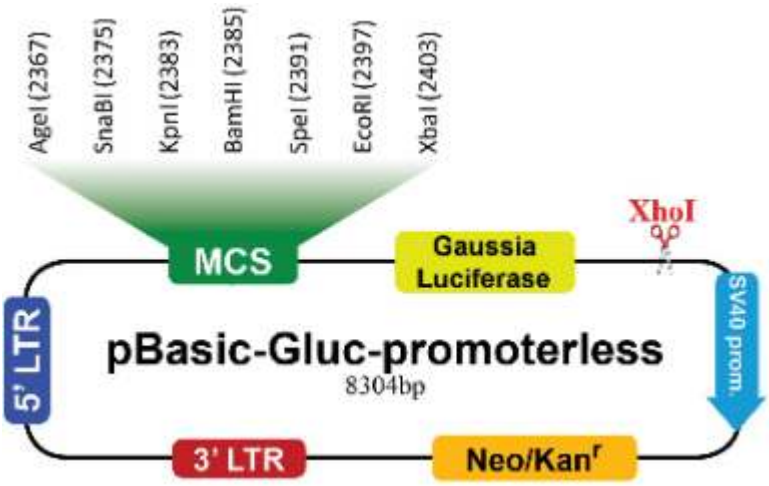
Plasmid map of the promoterless pLenti-Basic vector used for subcloning your promoter of interest:



Choice of three novel luciferase reporters:

- Gaussia luciferase
- Secreted Green Renilla luciferase
- Red-emitting firefly luciferase from the Italian firefly Luciola Italica
- Green-emitting firefly lcuiferase from Luciola Italica

Selection markers offered: Neomycin or Puromycin Plasmids can be propgated in bacteria using kanamycin.

References citing luciferase reporters in our LentiGlo and LentiPower products:

Gaussia Princeps Luciferase

1. Tannous BA (2009) Gaussia luciferase reporter assay for monitoring biological processes in culture and in vivo. Nature Protocols 4, - 582 - 591 (2009)

2. Development of a Dual-Luciferase Reporter System for In Vivo Visualization of MicroRNA Biogenesis and Posttranscriptional RegulationJi Young Lee, Soonhag Kim, Do Won Hwang, Jae Min Jeong, June-Key Chung, Myung Chul Lee, and Dong Soo Lee. J. Nucl. Med., Feb 2008; 49: 285 - 294.

3. Sensitive In Vivo Detection of Primary T Cells Expressing Membrane-Anchored Gaussia Luciferase for the Study of Adoptive T Cell Immunotherapy in Murine Models of Malignancy. Renier J. Brentjens, Elmer Santos, Raymond Yeh, Krista La Perle, Ricardo Toledo-Crow, Yan Nikhamin, Blesida Punzalan, David Entenberg, Iana Aranda, Bleserene Punzalan, Steven Larson, and Michel Sadelain Blood (ASH Annual Meeting Abstracts), Nov 2006; 108: 3685

4. Wurdinger T, Badr C, Pike L, de Kline R, Weissleder R, Breakerfield X, and Tannous B (2008) A secreted luciferase for ex vivo monitoring of in vivo processes. Nature Methods - 5, 171 - 173

5. Jinming Yang, Snjezana Zaja-Milatovic, Yee-Mon Thu, Francis Lee, Richard Smykla, and Ann Richmond (2009) Molecular determinants of melanoma malignancy: selecting targets for improved efficacy of chemotherapy. Mol. Cancer Ther., Mar 2009; 8: 636 - 647.

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Firefly luciferases from Luciola Italica

Note: The red- and green-emitting Luciola luciferases are improved significantly brighter versions (1000-fold improvement over the native Luciola luciferases) of the luciferases mentioned in the references below

1. Visualizing fewer than 10 mouse T cells with an enhanced firefly luciferase in immunocompetent mouse models of cancer Brian A. Rabinovich, Yang Ye, Tamara Etto, Jie Qing Chen, Hyam I. Levitsky, Willem W. Overwijk, Laurence J. N. Cooper, Juri Gelovani, and Patrick Hwu PNAS, Sep 2008; 105: 14342 - 14346.

2. "A Redshifted Codon-Optimized Firefly Luciferase is a Sensitive Reporter for Bioluminescence Imaging," H. Caysa, R. Jacob, N. Müther, B. Branchini, M. Messerle and A. Söling, Photochemical and Photobiological Sciences, 8: 52-56 (2009).

3. "Spectral-Resolved Gene Technology for Multiplexed Bioluminescence and High-Content Screening," E. Michelini, L. Cevenini, L. Mezzanotte, D. Ablamsky, T. Southworth, B. Branchini and A. Roda, Analytical Chemistry 80: 260-267 (2008).

4. "Combining Intracellular and secreted bioluminescent reporter proteins for multicolor cell-based assays," E. Michelini, L. Cevenini, L. Mezzanotte, D. Ablamsky, T. Southworth, B. R. Branchini and A. Roda, Photochem Photobiol Sci. 7: 212-217 (2008).

5. "Development of a Multiplexed Bioluminescent Cell-based Assay wit the Luc Gene from Luciola italica for High Throughput Screening of Cholesterol-Lowering Drugs." E. Michelini, T. L. Southworth, D. Ablamsky, B. R. Branchini and A. Roda, in Proceedings of the 14th International Symposium on Bioluminescence and Chemiluminescence: Chemistry Biology and Applications, San Diego, CA, October 15-19, 2006, edited by A. A. Szalay, P. J. Hill, L. J. Kricka and P. E. Stanley, World Scientific Publishing Co. Pte. Ltd., Singapore, pp. 119-122, 2007.

6. A redshifted codon-optimized firefly luciferase is a sensitive reporter for bioluminescence imaging Henrike Caysa, Roland Jacob, Nadine Müther, Bruce Branchini, Martin Messerle and Ariane Söling, Photochem. Photobiol. Sci., 2009, 8, 52

7. Improved red-emitting firefly luciferase for biotechnical applications. Audrey Davis, Connecticut College, 2009 . Can be accessed at the following link. digitalcommons.conncoll.edu/chemhp/5/

8. "Thermostable red and green light-producing firefly luciferase mutants for bioluminescent reporter applications," B.R. Branchini, D.M. Ablamsky, M.H. Murtiashaw, L. Uzasci*, H. Fraga and T.L. Southworth, Analytical Biochemistry, 361 (2): 253-262 (2007).

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Related resources

Poster presentation :

Applications fo Gaussia luciferase for imaging of stem cells using LentiGlo. View poster presentation link on our website under “Technical Resources”

http://www.targetingsystems.net/technical-resources.html

Technology spotlight empahising use of Dr Branchini’c red-emitting luciferase for tumor imaging. The red-emtting Luciola luciferase offered by us has been originally licensed from Dr Branchini’s lab (Connecticut College) but is an improved version being about a 1000-fold brighter than the native Luciola luciferase

http://www.rsc.org/Publishing/Journals/cb/Volume/2009/1/Light_up.asp

Licensing:

Note: All LentiPower vectors are sold for research purposes only and covered by multiple patents. For information regarding licensing issues please contact technology transfer by email info@targetingsystems.net or calling 1-866-620-4018.

Any commercial use pertaining to the Gaussia luciferase gene or commercial users using Gaussia luciferase-based products should directly contact Li Bryan at Prolume Inc, Arizona (928-367-1200)

Turbo Red 635 (Katushka) is a proprietary protein licensed from Evrogen Inc, Russia and commercial use of the same would require a license from Evrogen.

All other luciferases are covered by multiple patents held-licensed by Targeting Systems .

For enquiries relating to this product call tech support at 1-866-620-4018