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Targeting Systems Introduces :

LentiPower™

Pluristem Innovations

Lenti-luciferase technology to study gene expression in live cells. Harness the power of our novel Lenti-luciferase technology to study gene expression in live cells. Speed up research and increase assay sensitivity.

With LentiPower you can

- Efficiently and stably transduce both dividing and non-dividing cells in a reproducible manner.
- Choose the most physiologically relevant promoter for your applications
- Study long term gene expression
- Study gene expression in living cells using a combination of novel secreted luciiferases and bright fluorescent proteins.
- Harness the power of Lenti-luciferase technology to achieve highest transfection efficiencies even in the most difficult cell types such as stem cells and neuronal cells.
- Create stably transfected cells almost instantaneously.
- Study gene expression in live cells .
- Choose from a panel of novel secreted luciferase reporters - the brightest and the best
- Analyze multiple pathways in the same group of stably transfected cells using a panel of diverse luciferase reporters with different subrates and emission maxima

Efficient , stable gene expression in any cell type coupled with powerful expression.

Perform your studies in the cells you want, at the expression levels you need

Using the LentiPower system you will produce non-replicating viral particles that can efficiently transduce nearly any dividing or non-diving cell type. Lentivirus mediated expression allows reproducible, precise control over both the number of stably transfected cells and the number of stably transfected copies of your gene.

The results speak for themselves...

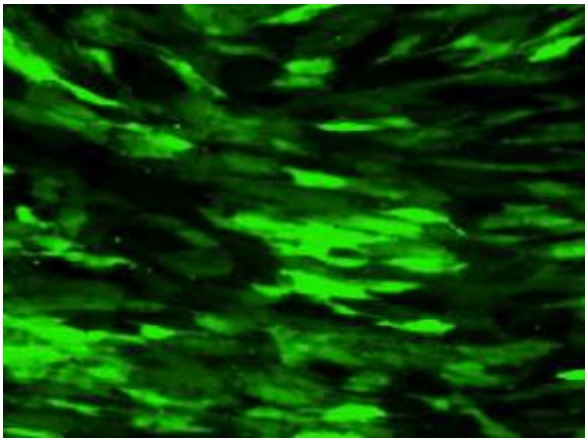

Figure 1: Primary human mesenchymal stem cells transduced with a lentivirus vector expressing both Green fluorescent protein and Gaussia luciferase (transfection efficiency almost 100%)

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What is LentiPower and how does it work

LentiPower™ enables you to make your own lentiviral particles quickly and efficiently by packaging our Lenti-luciferase vectors with a packaging mix and produce recombinant lentivirus. We also provide promoterless Lenti plasmids with multiple cloning sites upstream of the luciferase gene which enable you to subclone your promoter of choice upstream of the luciferase gene in the Lenti plasmid so that you can now produce a lentivirus expressing luciferase under control of your promoter of interest. The steps involved in makign a ready to use lentivirus in HEK-293 cells by co-transfection of the Lenti-luciferase plasmids along with a packaging mix (provided as par to the LentiPower system) are outlined below.

1. Make the plenty construct containing your promoter of interest.
2. Co-transfect 293 cells with your plenti Expression vector and the Lenti packaging mix
3. Harvest cell supernatant and determine the titer
4. Transduce cells, select for stable transfection and assay.
5. Alternately sort out transfected cells using FACS
6. Store up to one year

Flexible Options for cloning and choosing ultra-sensitive luciferase reporters:

Choice from several available improved novel luciferase reporters

- Gaussia Princeps luciferase- The brightest known secreted, luciferase, emission max 482 nm.
- Red–emitting firefly luciferase from Luciola Italica (emission max 617 nm)
- Green-emitting, secreted mutant of Renilla luciferase, (emission max 527 nm)- the brightest of our luciferase reporters for intracellular expresssion

Choose between two fluorescent reporter proteins:

- Turbo red 635 (popularly known as Katushka). Katushka is one of the brightest red-emitting fluorescent proteins for in vitro and in vivo imaging applications. It´s emission max (655 nm) offerssignificant advantages for imaging deep-seated tissues or cultured cells.
- The second choice of a reporter protein is EGFP which emits in the green region.

Choose between two promoters for constitutive gene expression:

Different promoters work differently in various cell lines.

- CMV promoter for high levels of expression in several cell lines.
- Ubiquitin promoter- For efficient in vivo expression or when using cell lines that downegulate the CMV promoter.

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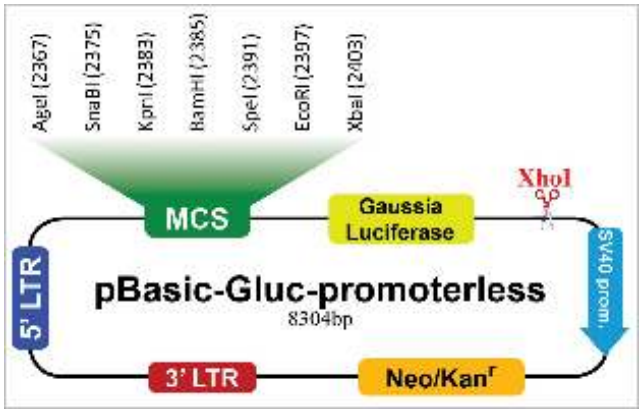
LentiPower Basic (promoterless) vectors expressing luciferase alone

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

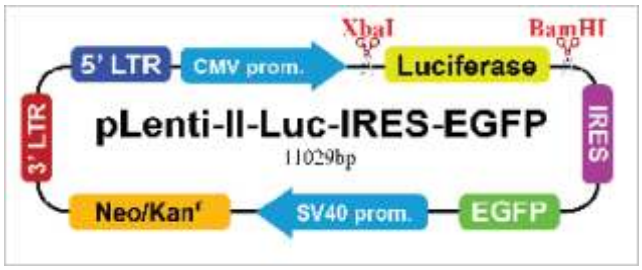


Choice of three novel luciferase reporters:

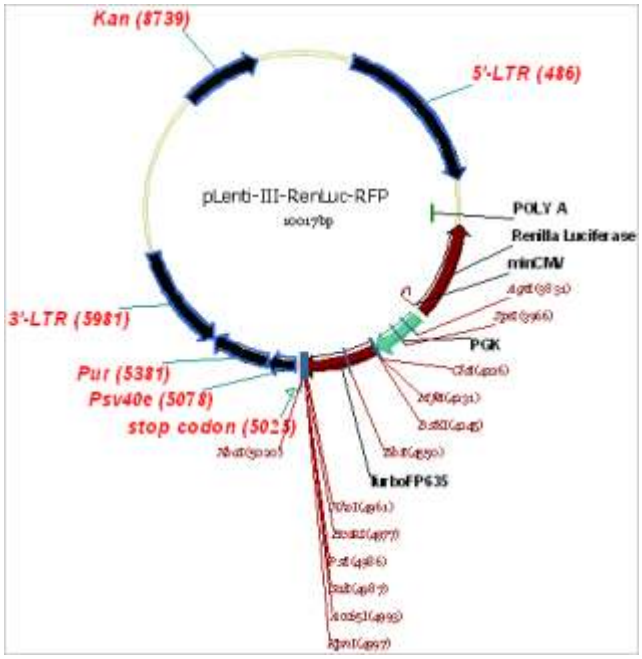
- Gaussia luciferase
- Secreted Green Renilla luciferase
- Red-emitting firefly luciferase from the Italian firefly Luciola Italica
- Green-emitting firefly lcuiferase from Luciola Italica

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

LentiPower Control vectors expressing luciferase along with fluorescent proteins for easy visualization of transfection efficiency



Option 1 - **The fluorescent protein is expressed using an IRES plenti-CMV-GLuc-IRES-GFP:** Lenti vector expressing Gaussia luciferase under control of the CMV promoter using an IRES to express EGFP. Other luciferase options available under this format include red-emitting firefly luciferase and GFP



PLenti.CMV-GrRenLuc-RFP : Our latest contol lenti plasmid encoding a secreted green-emitting Renilla luciferase under control of the CMV promoter and Turbo Red 635 (Katushka) under control of the phosphoglycerokinase promoter.

Selection markers offered : Puromycin
Plasmid can be amplified in bacteria using kanamycin

Other options:

plenti-CMV-grRenLuc: the same as the above Lenti plasmid but expressing Green-emitting Renilla luciferse alone without RFP

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Add your promoter or create additional modifications- Expression vectors to meet any experimental design

Specialized applications such as gene expression in stem cells, primary cells, animal models or tissue –specific gene expression require the user to express the gene of interest under a physiologically relevant promoter. Other applications require co-expressing a second gene of interest , making fusions of the reporter gene or expressing RNAi to study gene silencing. To allow a greater degree of flexibility to allow these specialized applications, the LentiPower plasmids have been given specialized features. Some of these are listed below:

A Basic (promoter-less) Lenti-plasmid vector which has a multiple cloning site with a lot of unique restriction sites upstream of the luciferase reporter gene to allow for greater compaitibility to subclone the promoter of interest

A reporter fluorescent protein with unique restriction sites for fusing target genes with the fluorescent reporter.

Options for subcloning an RNAi expressing gene to co'express siRNA against the target gene along with the luciferase reporter, instead of the fluorescent protein reporter gene.

Please contact our tech support at 1-866-620-4018 for details on specialized applications

Summarized below

- Efficiently and rapidly clone both promoter and a gene of interest into a LentiPower Lentiviral expression vector
- Choose the most physiologically relevant promoter for your cell type by expressing your gene of interest from multiple promoters constructs
- Make fusion proteins in which the gene of interest is fused to a fluorescent protein reporter in the LentiPower expression vector to enable visualization of expression patterns and cellular localization of the expressed protein
- Study tissue-specific gene expression

Designed with increased safety in mind:

Key safety features are built into the LentiPower Lentiviral System. The gene delivery vector is safe and is far removed from the wild-type virus (since majority of viral proteins have been removed). The packaging functions are supplied in trans, i.e on other packaging plasmids.

- Viral particles generated are replication-incompetent and carry the gene of interest- no other viral particles are produced.
- Transduced and integrated lentiviral vectors are no longer capable of producing a packageable viral genome
- Safe powerful expression at an economical price

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 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.

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Ordering Information

Catalog No.	Name of LentiPower plasmid	Description	Price
LP-01	PLenti-Basic-Gluc	Promoterless Lenti plasmid expressing Gaussia luciferase, packaging mix, 1000 assays of luciferase assay reagent	$1650
LP-02	PLenti-CMV-Gluc	Control Lenti plasmid expressing Gaussia luciferase under control of the CMV promoter, packaging mix, 1000 assays of luciferase assay reagent	$1650
LP-03	pLenti-CMV-GLuc-IRES-GFP	Control Lenti plasmid expressing Gaussia luciferase under CMV promoter and EGFP using an IRES, packaging mix, 1000 assays of lcufierase assay reagent	$1650
LP-04	pLenti-BasicFLuc	Promoterless Lenti plasmid expressing Firefly luciferase, packaging mix, 1000 assays of luciferase assay reagent	$1650
LP-05	pLenti-CMV'RedFluc-IRES-EGFP	Control Lenti plasmid expressing Red firefly luciferase under control of the CMV promoter and and EGFP using an IRES	$1650
LP-06	pLenti-CMV-GrRenLuc	Control Lenti plasmid expressing Green Renilla luciferase under CMV promoter	$1650
LP-12	PLentiCMV-GrRenLuc-RFP	Control Lenti plasmid expressing Green Renilla luciferase under control of CMV promoter and RFP under phosphoglycerokinase promoter	$1650

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

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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.

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).