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New Products

Introducing Red and Green Luciferases from Luciola Italica

Applications:

- Single and multiplexed Luciferase assay systems for high throughput screening.
- Mammalian expression vectors for studying regulation of gene expression

The demand for multiplexed reporter systems to draw parallels between multiple parameters within the same cell is ever increasing. Because high throughput and ultra-high throughput screening has become the norm in the Pharmaceutical research facilities, the ability to draw correlations in low volume assays has created a trend towards fully automated miniaturized assays using 384 well and 1536-well formats. These requirements have created a need for assays that require no intervention form the researcher during screening, minimize number of handling steps and have the ability to deliver consistent results in single micro liter volumes.

The first requirement for applicability of a luciferase reporter for screening applications is sensitivity (increased brightness), stability of the bioluminescent signal over a long time, and an emission max that would allow multiplexing with other luciferase reporters. Clearly a greater the number of luciferase reporters would allow one to analyze regulation of multiple promoter activities in the same sample of transfected cells without significantly increasing the number of handling steps or assay time. It is to meet these requirements that Targeting Systems has developed a series of novel luciferase reporters with different emission maxima that are suitable for multiplexed assays.

The red emitting and green emitting luciferase reporters from the Italian firefly Luciola Italica are particularly attractive for multiplexing with Gaussia luciferase (licensed from Prolume Inc, AZ), the first luciferase reporter developed by Targeting Systems . The three luciferase reporters are described below:

Gaussia Princeps luciferase, is a secreted protein that provided enhanced sensitivity over the existing reporters as well as affords the opportunity for time resolved analyses. The significantly brighter Gaussia Princeps luciferase (1000 fold brighter than Photinus/Renilla) allowed the researcher to explore temporal gene expression patterns in single plates of cultured cells through repeated sampling and assaying of the culture media.

A red-emitting luciferase and a green-emitting lcuiferase from the Italian firefly Luciola Italica. The emission wavelengths (see below table) of these Luciola luciferases (green 550 nm, red 609 nm) and their enhanced sensitivity compared to Photinus (green 10-times brighter, red 100 times brighter) facilitated assay multiplexing by incorporating these luciferases with the Gaussia luciferase (emission max 482). The Luciola luciferases use firefly luciferin as a substrate and require ATP and magnesium.

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Principle of the Luciferase Assay

The luciferase assay kit is based upon the bioluminescent measurement of firefly luciferase. This enzyme catalysis the formation of light from ATP and luciferin according to the following reaction:

$$\text{ATP + Luciferin + O2} \xrightarrow[\text{Mg++}]{\text{Luciferase}} \text{Oxyluciferin + AMP + PPi + CO2 + LIGHT}$$

The intensity of light emission is linearly related to the amount of luciferase and is measured using a luminometer. Luciferase is the most widely used genetic reporter in studies on gene expression due to it's high sensitivity dynamic range and its natural absence from mammalian cells. The luciferase assay kit offered by Targeting Systems offers the advantages of high sensitivity, consistent reproducibility and cost effectiveness along with the added convenience of a one step assay. The FLAR-1 luciferase assay reagent has been formulated with the cell lysis components, the luciferrin substrate and the bioluminescence stabilizers all included in a single assay reagent.

Typically the FLAR-1 reagent has been formulated for assaying luciferase activity cells grown in the 96-well format It is recommended that for optimum performance cells are cultured in 100µl volumes in bioluminescence compatible white walled, clear bottomed 96 well plates. Before Luciferase expression can be measured by bioluminescence, the enzyme must first be extracted from the cells. The reconstituted Luciferase assay kit plus lysis buffer) facilitates the simultaneous extraction and measurement of luciferase from mammalian cells. For optimal convenience the kit can be used in conjunction with automatic reagent dispensing facilities, however this is not an essential requirement of this assay, as a multichannel pipette capable of dispensing 100µl may be used for the addition of reconstituted Luciferase Detection Reagent. The luciferase assay kit is intended for the detection of the expression of firefly luciferase and is not suitable for the assay of luciferase from other origins.

Specifications:

Linear range- Assay linear over seven orders of magnitude
Limit of detection – less than 1 fg of luciferase per sample

Product Profile: Targeting Systems offers a one-step luciferase assay kit to facilitate measurement of luciferase activity in HTS (high throughput applications). Note that the kits supplied by Targeting Systems are one step assay kits, i.e. the reagent has all the ingredients necessary for lysing cells as well as the luciferin substrate and stabilizers of the luciferase reaction in a single solution unlike most other kits where the assay buffer has to be mixed with the luciferin substrate provided in a separate bottle.

Luciferase Assay Kit (FLAR-1): This kit can be conveniently used for measuring luciferase activity in 96-well plates (or in preparations of lysed cells). As seen in the figure below, the bioluminescent signal is quite stable with a half life greater than

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60 mins. The FLAR-1 luciferase assay kit can also be used to measure luciferase activity in prelysed cell extracts. In this case 5-50 µl of the cell lysate is mixed with 100 ul of the luciferase FLAR-1 assay reagent, mixed well and read immediately in the luminometer. The FLAR-1 reagent includes several stabilizers of the luciferase enzyme in its composition. Since the stabilizers used are not luciferase inhibitors a stable bioluminescent signal

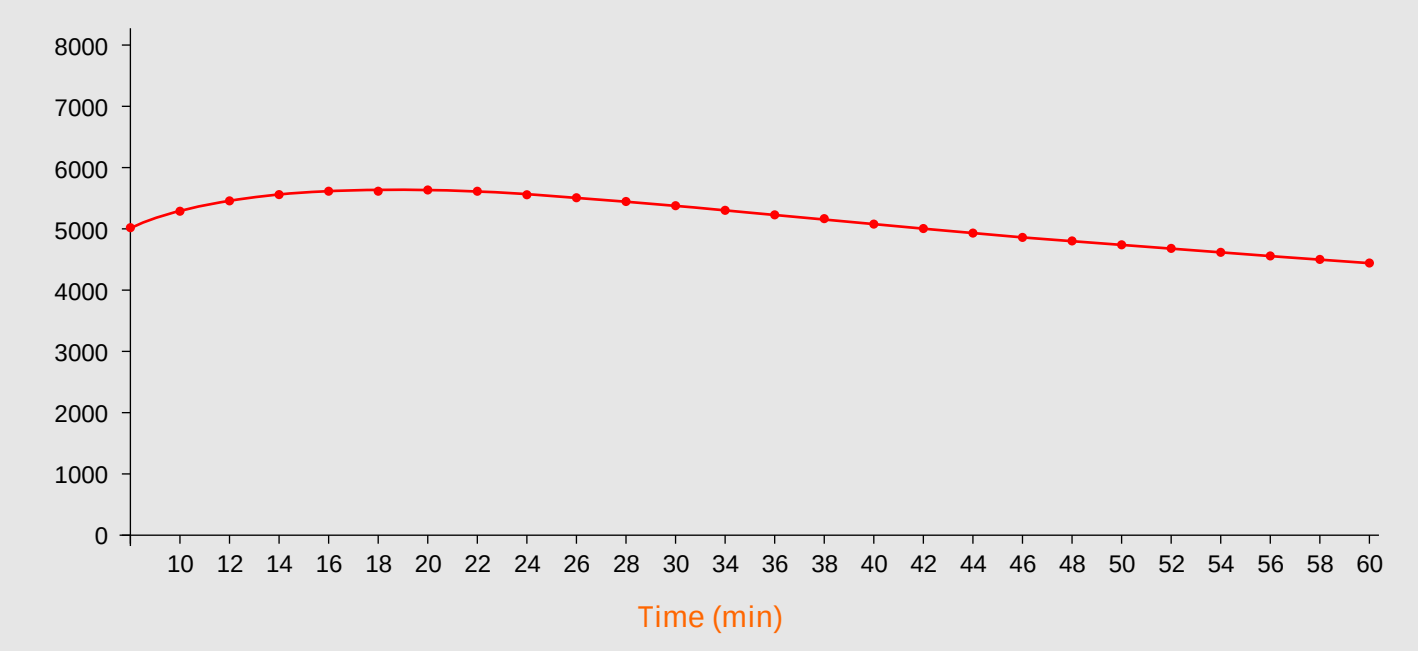

Figure 1: Stability of the bioluminescent signal using the FLAR luciferase assay reagent). Results are expresses as the mean of quadruplicate determinations (CV less than 5%). The intensity of the bioluminescent signal using the FLAR reagent from Targeting Systems is comparable to the intensity of the bioluminescent signal in assays using Promega's Bright glo reagent. In the experiment shown above the FLAR reagent was directly added to the supernatant cell culture media

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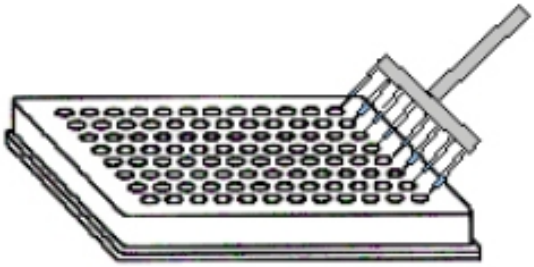
Protocol:

ASSAY IN 96-WELL FORMAT: Add 100 µl of the FLAR-1 reagent to 50 µl or 100 µl of the supernatant culture media from cells grown in multi-well tissue culture dishes. Incubate for at least 10 minutes and read in a lumionometer or micro plate reader.

Assay Of Luciferase Activity In Cell Lysates: The FLAR-1 luciferase assay reagents can also be used to measure luciferase activity in pre-lysed cells. Mix 5-50 µl of luciferase containing sample or cell lysate with 100 µl of the luciferase assay kit (TS-1) and read immediately in the luminometer. All assay reagents should be close to room temperature at the time of assay.

NOTE: If you need to lyse cells first use the cell lysis buffer from Targeting Systems or the Cell culture lysis reagent from Promega (E1531).

Simple One-step Assay



Aspirate cell culture media

Add Firefly Luciferase Assay
Mix And Read

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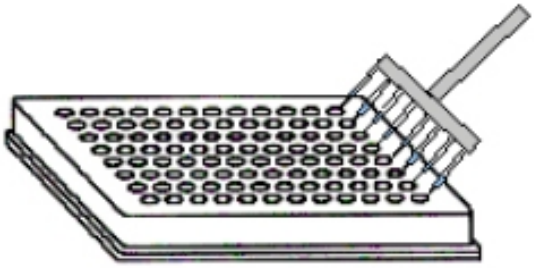
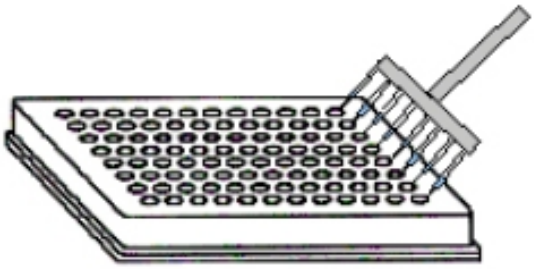
Optional Two-step Assay



Lyse Cells

Add Firefly Luciferase Assay Mix And Read

Lyse Cells Using 1x Cell Lysis Buffer (30 µl Per Well) From Targeting Systems Or Promega (cat# 1531)



Add Firefly Luciferase Assay Mix (100 µl Per Well) And Read

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Firefly Luciferase Assay System Flar-1

Product	Cat. #	Size	Unit Price
Luciferase assay kit	FLAR-1000	100ml	$300
FLAR-1	FLAR-1L	1000ml	$2500

Special discounts available for bulk purchasesProduct

DESCRIPTION: The luciferase assay kit FLAR-1 is a homogenous luciferase gene detection system. Targeting Systems has two versions of a one-step luciferase assay kit to facilitate measurement of luciferase activity in HTS (high throughput applications). Note that the kits supplied by Targeting Systems are one step assay kits, i.e. the reagent has all the ingredients necessary for lysing cells as well as the luciferin substrate and stabilizers of the luciferase reaction in a single solution unlike other kits where the assay buffer has to be mixed with the luciferin substrate provided in a separate bottle.

The FLAR-1 luciferase assay kit can be used to measure luciferase activity in pre-lysed cell extracts or it can be added directly to the cells (see attached protocol sheet). The assay is compatible with both sample processing robots and with reagent injectors in many luminometers

Specifications:

- Reproducibility – CV less than 5%
- Linear range- Assay linear over seven orders of magnitude
- Limit of detection – less than 1 fg of luciferase per sample
- No disposal problems or hazards are associated with the use of these luciferase assay kits

Storage: Store reagents at –20° C or –70° C

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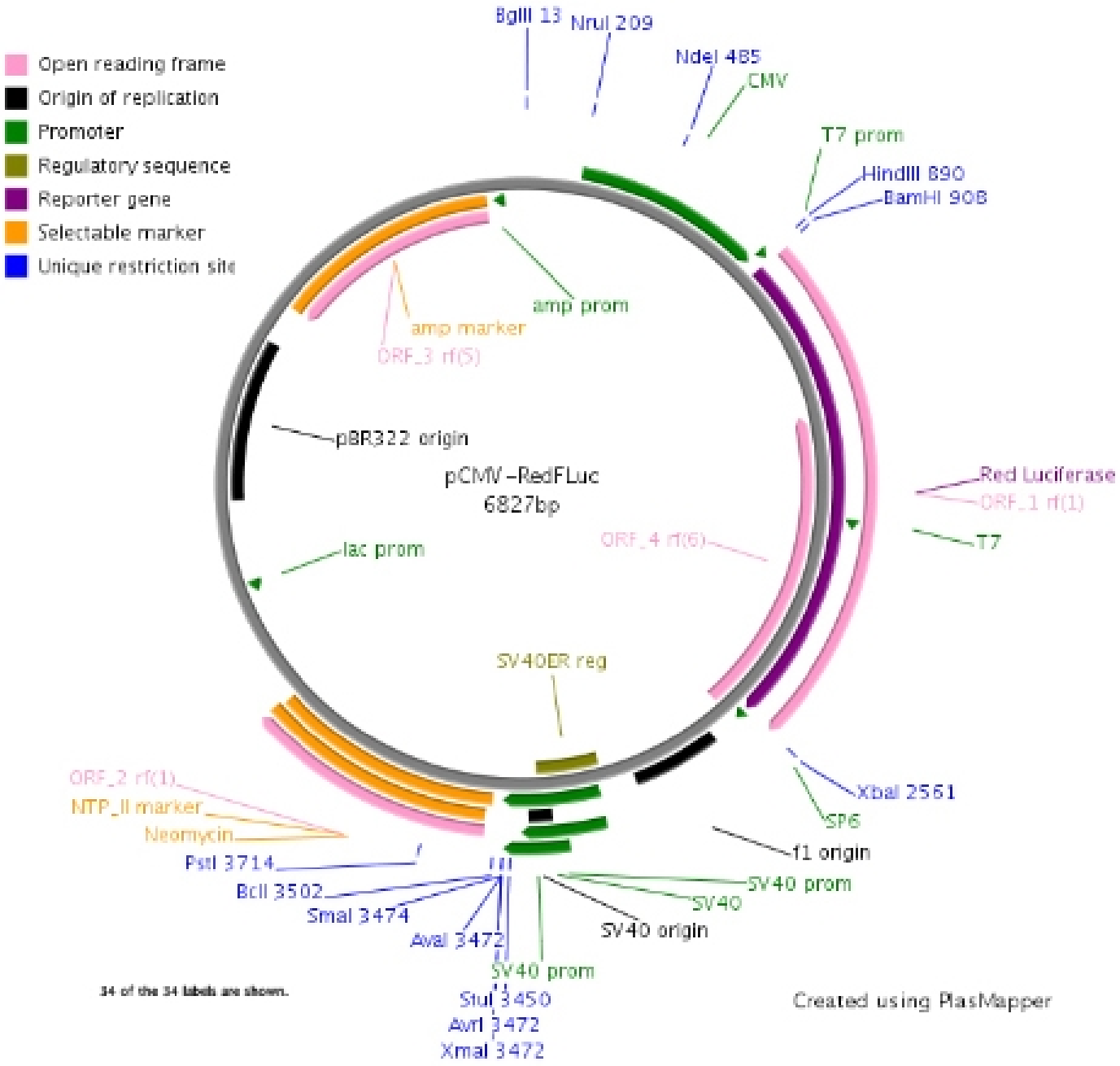
Expression vectors expressing the firefly luciferases from Luciola Italica

pCMV-Red-Fluc- Expression vector expressing Red-emitting luciferase under control of CMV promoter



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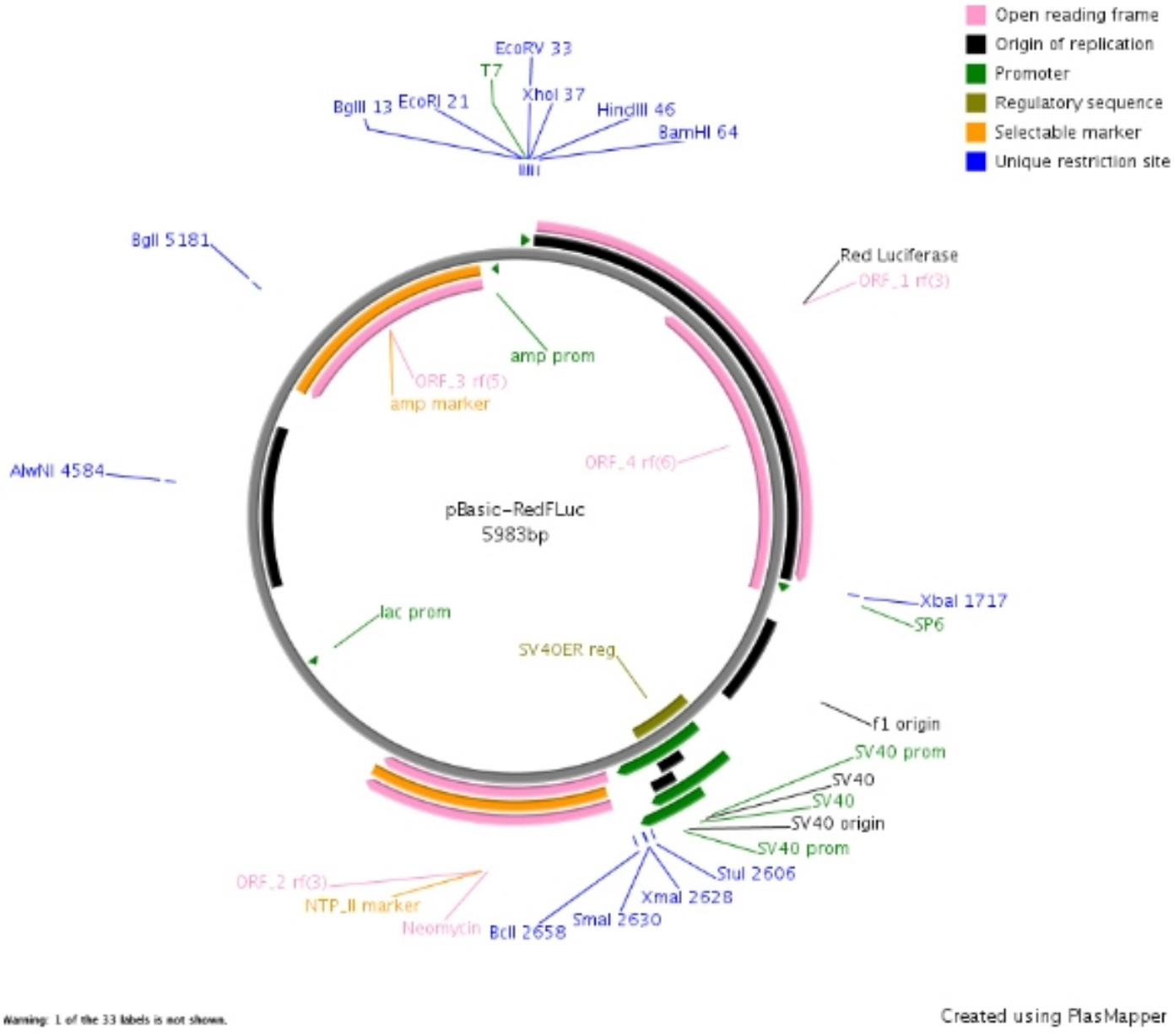
pBasic-Red-FLuc Expression vector expressing Red-emitting luciferase under control of CMV promoter



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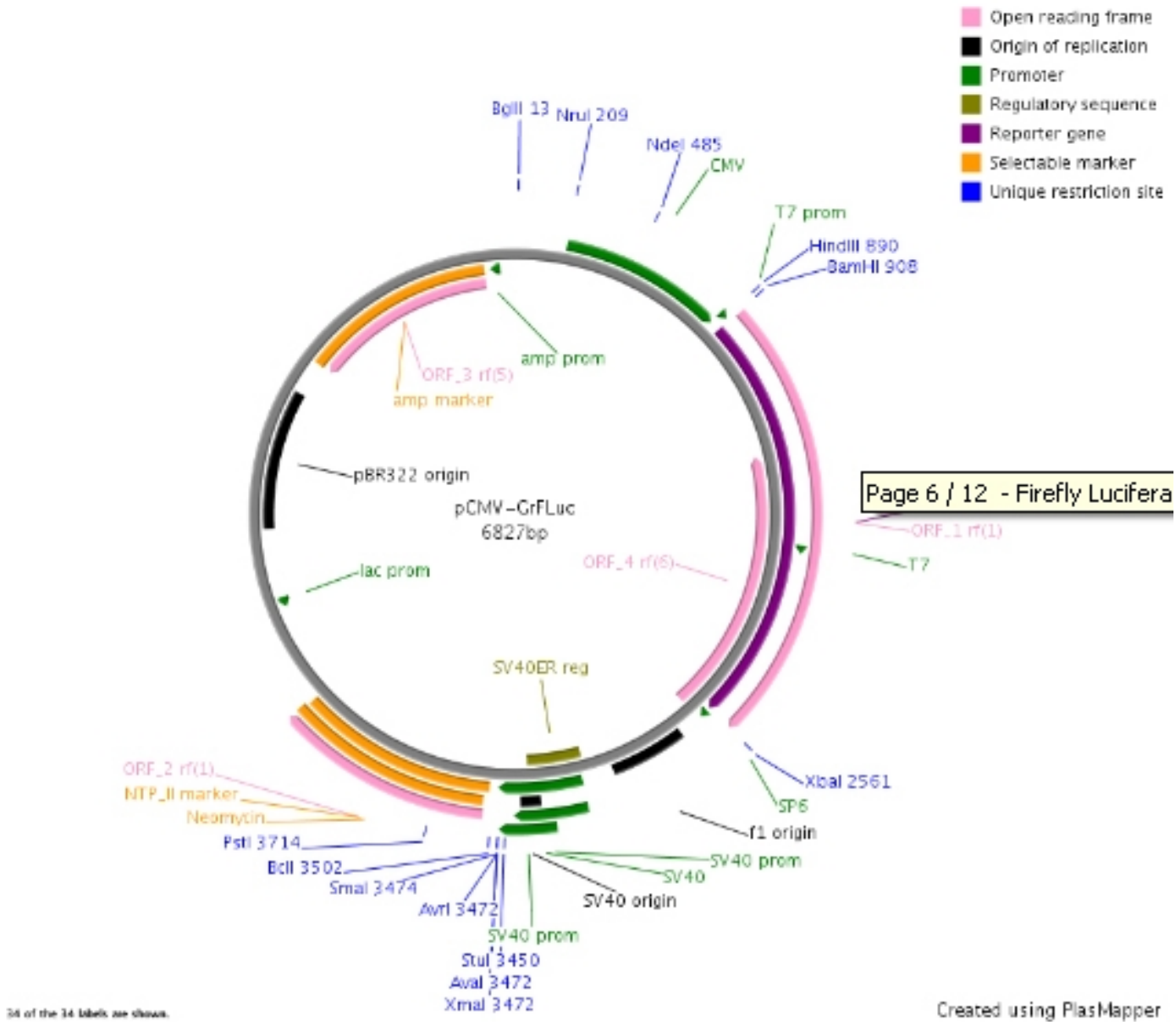
pCMV-Gr-Fluc- Expression vector expressing green-emitting luciferase under control of CMV promoter



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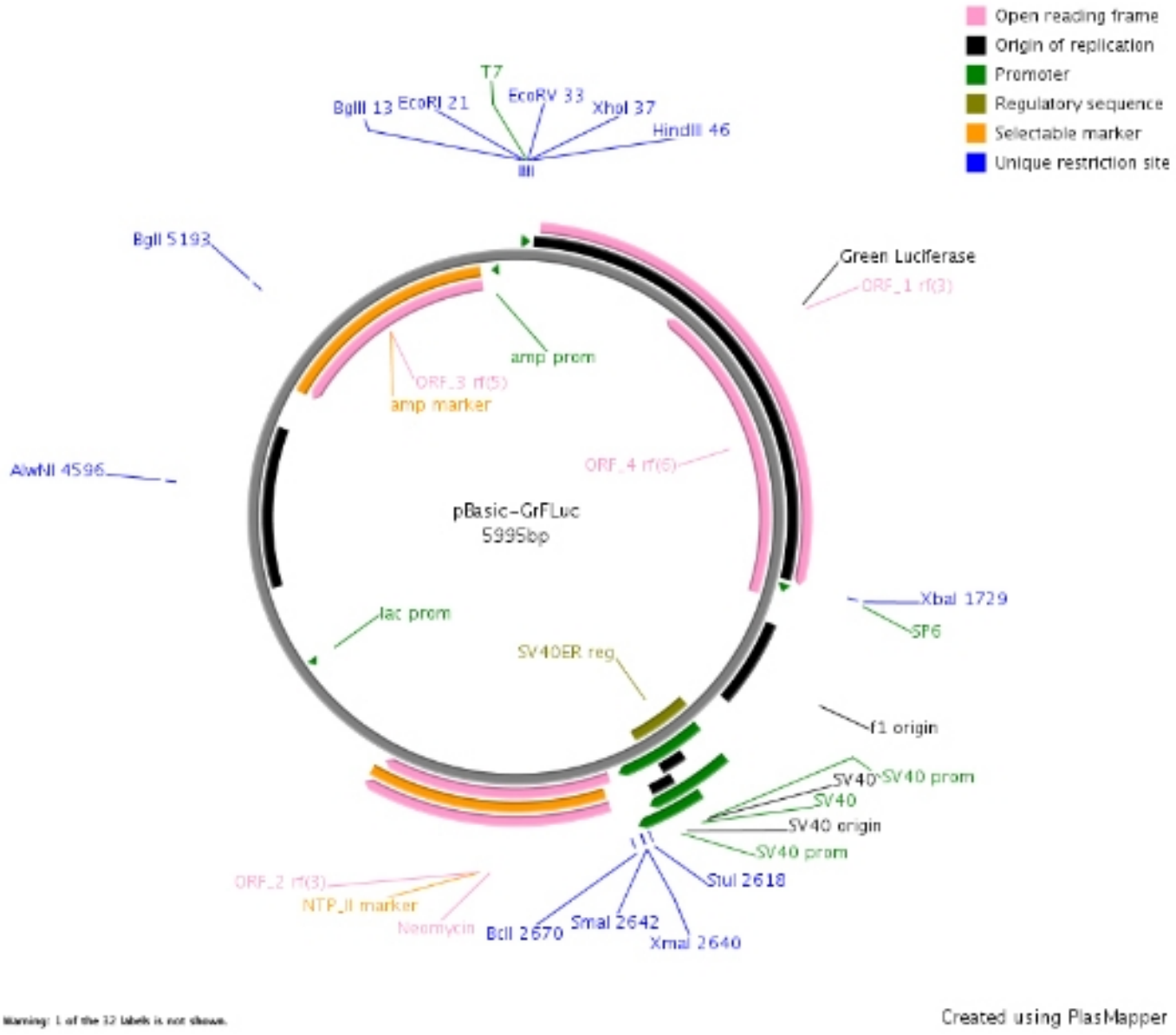
pBasic-GrFluc Expression vector expressing Red-emitting luciferase under control of CMV promoter



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The plasmid map shown above was constructed with the help of the following link:

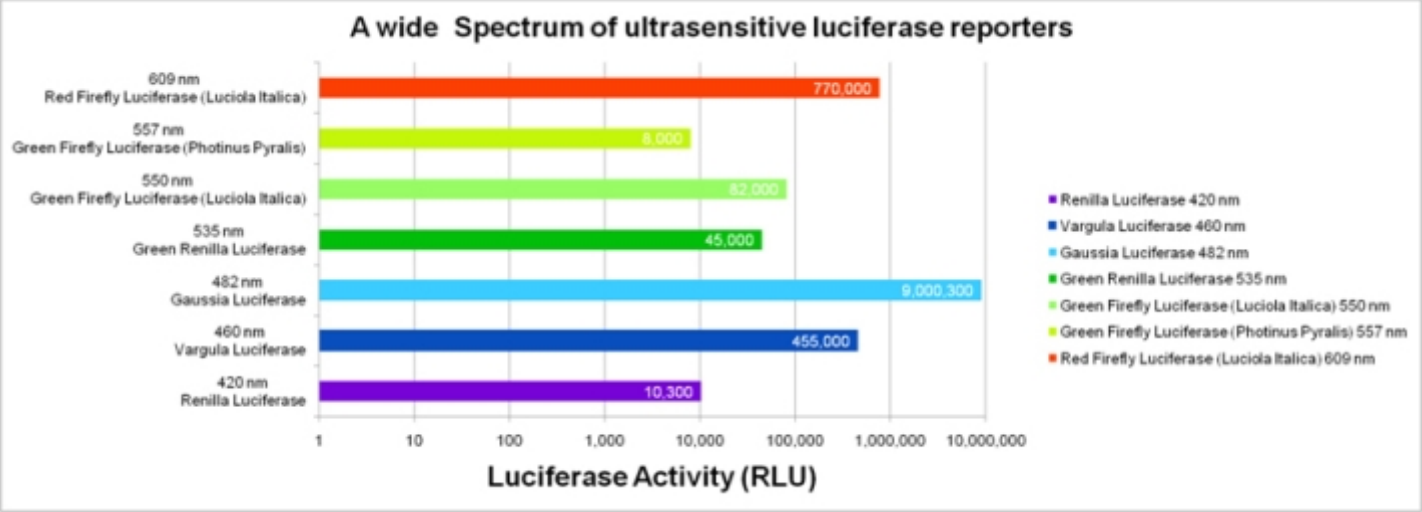
http://wishart.biology.ualberta.ca/PlasMapper/jsp/displayPlasmidMap.jsp?fileName=plasMap115_1101977745436.jpg&fileFormat=jpgXiaoli Dong, Paul Stothard, Ian J. Forsythe, and David S. Wishart "PlasMapper: a web server for drawing and auto-annotating plasmid maps" Nucleic Acids Res. 2004 Jul 1;32(Web Server issue):W660-4.

The CMV promoter is covered by U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

Gaussia Luciferase is covered by US patent #6,232,107 and IPO patents issued to Prolume Inc. This Plasmid is being sold for research purposes only. Commercial users/ For profit companies and foundations will require a license from Prolume (www.nanolight.com) for the use of Gaussia gene after 180 days of evaluation and from the University of Iowa Research Foundation for the use of the CMV promoter.

These products are sold for research use only. Commercial use of these products requires licenses from Targetimg Systems. Luciferase assay reagents and expression vectors are covered under the following pending patents:

Patent number US2007/0190587). and Wo2006096735.



A comparision of the Luciola Italica red and green-emitting luciferasese with other luciferase reporters.:

Please note that the Photinus luciferase used in this experiment was the PGL3 series Photinus luciferase subcloned under control of the CMV promoter The experiment was conducted in HEK-293 cells.using either Gaussia luciferase or Vargula luciferase as the denominator plasmid.

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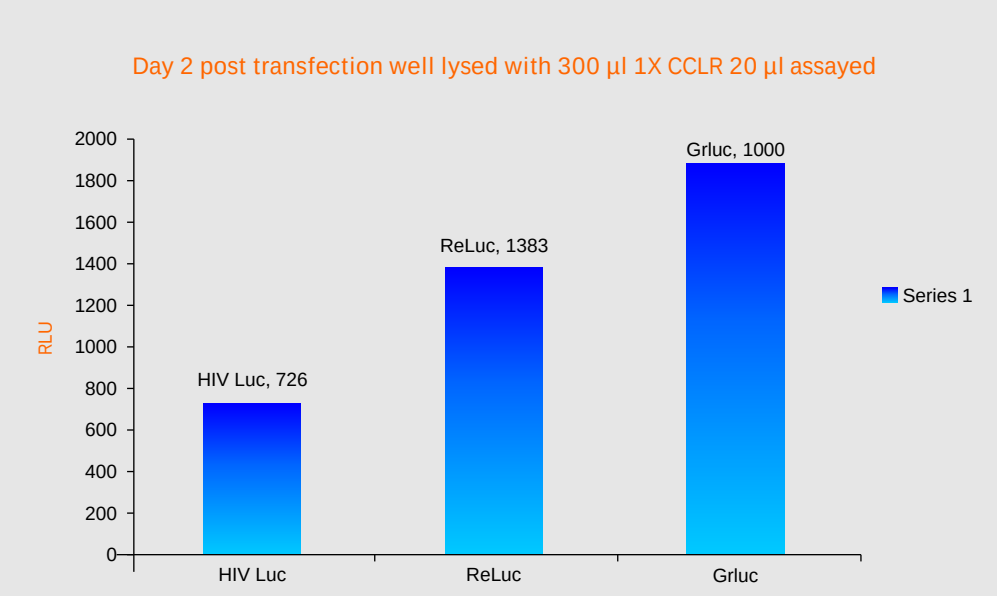
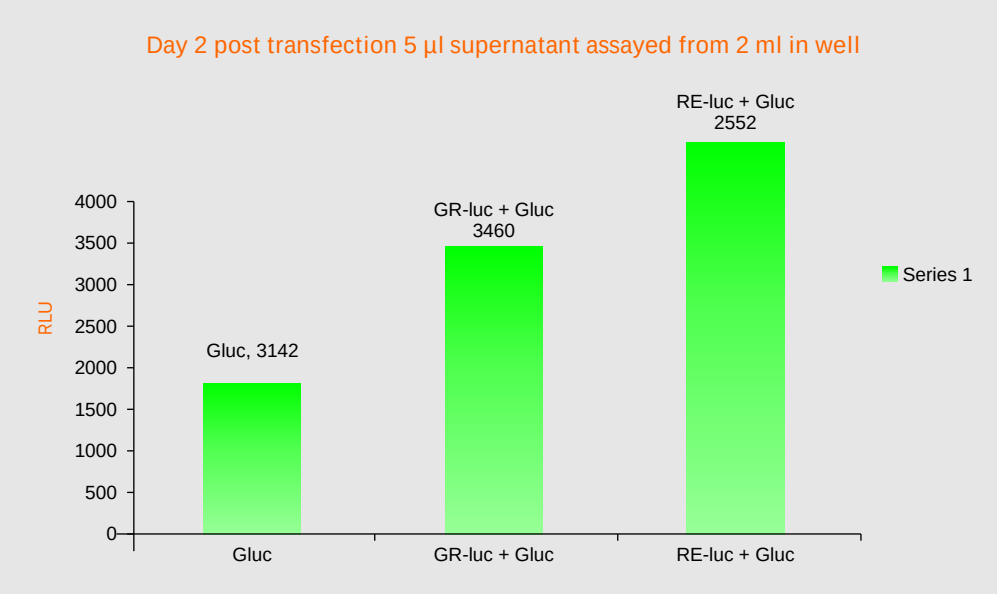
A Dual Luciferase assay based on Gaussia luciferase and firefly luciferase as reporters: These can be performed either by assaying Gaussia luciferase activity in the supernatant and then measuring firefly luciferase activity in cell lysates *Figures A and Gaussia luciferase activity in supernatants (Fig B) or by measuring both Gaussia luciferase and red-emitting firefly luciferase in cell lysates (data not shown). Users wishing to use the Gaussia-firefly luciferase dual assay system on cell lysates should use the pCMV-Gluc-Kdel/pGluc-Basic-kdel vectors as these give more efficient intracellular expression of Gaussia luciferase.





For an overview of luciferase-relateed products please access the following link on our website:
http://targetingsystems.net/drug-discovery.html

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The different types of single and multiplexed luciferase reporter assays we offer are listed below:

A major advantage of our multiplexed assays (TLAR-1) , TVLAR-1 is that three different luciferases can be assyed with a single solution using different filters to spectrally resolve the luciferase acitivties. We have the expertise to enable trasnfection of 5 different genes into a single cell and analyse gene expression of 5 different genes in the same well of transfected cells.

Gaussia luciferase reagent GAR-1 - Assay reagent for standard assays (brighter)

Gaussia luciferase reagent GAR-2 (#0090A) – Assay reagent for high throughput applications (a more stable luminescent signal)

Gaussia luciferase assay reagent GAR-B2 : Gives the brightest signal suitable for in vivo Gaussia luciferase activity sampling blood or urine samples

Firefly luciferase assay reagent (FLAR-1)- Assay reagent for assaying Firefly luciferase. A homogenous one step assay format suitable for high throughput applications (30% decay in 2 hours). Also suitable for dual assay of both Luciola luciferases Also download the firefly luciferase product brochure for more info.

Vargula (Cypridina) luciferase assay reagent (VLAR-1)

Assay reagent for Vargula luciferase- based assays, Download the Vargula luciferase product brochure for more info

Multiplexed Assays (Dual/Triple reporter) involving Gaussia Luciferase:

TLAR-1 – A single solution-based triple lucfierase reporter assay involving Gaussia luciferase with red and green Luciola luciferases. Also useful as a triple luciferase assay reagent using Renilla luciferase in place of Gaussia luciferase. If only a dual luciferase assay is required we recommend using the TLAR-1 reagent to multiplex Gaussia luciferase (or renilla luciferase) with the Red-emitting Luciola luciferase

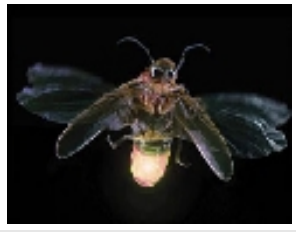
TVLAR-2 - A single solution-based triple luciferase reporter assay involving Vargula (Cypridina) luciferase with red and green Luciola luciferases. Also useful as a triple luciferase assay reagent using If only a dual luciferase assay is required we recommend using the TVLAR-2 reagent to multiplex Vargula luciferase with the Red-emitting Luciola luciferase

5X Cell lysis reagent: A passive cell lysis buffer compatible with assays for all of our

Other applications: The red-emitting luciferase from Luciola Italica appears very promising for in vivo imaging applications:

Light up your life processes, with more light…….
The red-emitting modified Luciola luciferase we offer is over 100- times brighter than Photinus luciferase. Now there is enough light to do things hard to do before…

Imaging of deep seated tissue such as brain tumors
Tracking implanted stem cells



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References:

For more info on the advantages of the red-emitting luciferase fro imaging applications please view the links below:

http://news.biocompare.com/News/NewsStory/287653/Targeting-Systems-Announces-New-Red-Emitting-Luciferase-Reporter-That-is-at-Least-100x-Brighter-for-Deeper-Tissue-Penetration.html

Citation: 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
DOI: 10.1039/b814566k

References citing luciferase reporter products from Targeting Systems:

1. 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.

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

3. Wurdinger T, Badr C and Tannous B (2008) Gaussia luciferase blood level as an index of cell growth and proliferation

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. Cheng G and Davis RE (2007). An improved and secreted luciferase reporter for schistosomes. Molecular & Biochemical Parasitology 155 (2007) 167–171.

6. Drug Discovery Today: Technologies Volume 1, Issue 4, December 2004, Pages 357-364

7. Nicola Ternette,1 Daniela Stefanou,1 Seraphin Kuate,1 Klaus Überla,1 and Thomas Grunwald (2007) Expression of RNA virus proteins by RNA polymerase II dependent expression plasmids is hindered at multiple steps. Virol J. 2007; 4: 51.

8. Guofeng Chenga,1, Leah Cohenb,1, Claudette Mikhli b, Marzena Jankowska-Anyszka c (2007) In vivo translation and stability of trans-spliced mRNAs in nematode embryos.

Relevant interesting papers on new applications of Gaussia luciferase:

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

10. 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 (2006) 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. Blood (ASH Annual Meeting Abstracts), Nov 2006; 108: 3685

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References citing red and green-emitting luciferases from the Italian firefly Luciola Italica:

Note: The red- and green-emitting Luciola luciferases are improved significantly brighter versions (mammalian expression) of the luciferases mentioned in the references below

1. "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).

2. "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).

3. "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).

4. "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.

5. 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

6. 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/

7. "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).

8. "Lucifease from the Italian firefly Luciola italica: Molecular cloning and expression," B.R. Branchini, T.L. Southworth, J.P. DeAngelis*, A. Roda, and E. Michelini, in Comparative Biochemistry and Physiology, Part B, 145, pp.159-167 (2006).

9. "NMR Assignment of the Backbone Resonances of the Firefly Luciferase C-Terminal 14.3 kDa Domain," B.R. Branchini, S.A. Gonzales, and R. Magyar in Journal of Biomolecular NMR, vol 33, p. 73 (2005).

10."Red- and Green-Emitting Firefly Lucifease Mutants for Bioluminescent Reporter Applications," B.R. Branchini, T.L. Southworth, N.K. Khattak*, E. Michelini, and A. Roda in Analytical Biochemistry, vol. 345, pp.140-148 (2005).

Some relevant interesting papers:

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. "Synergistic Mutations Produce Blue-Shifted Bioluminescence in Firefly Luciferase," B. R. Branchini, D. M. Ablamsky, J. M. Rosenman*, L. Uzasci*, T. L. Southworth and M. Zimmer, Biochemistry 46 (48): 13847-13855 (2007).