# *Gaussia princeps* luciferase

hGLuc

Arvind Chopra, PhD[1]

Created: January 31, 2008; Updated: February 28, 2008.

| | | |
|---|---|---|
| **Chemical name:** | *Gaussia princeps* luciferase | |
| **Abbreviated name:** | GLuc | |
| **Synonym:** | *Gaussia* luciferase | |
| **Agent Category:** | Luciferase | |
| **Target:** | Non-targeted | |
| **Target Category:** | Photon emission/bioluminescence | |
| **Method of detection:** | Optical imaging | |
| **Source of signal:** | Photons | |
| **Activation:** | No | |
| **Studies:** | • *In vitro*<br>• Rodents | Click here for the protein and nucleotide sequence of *G. princeps* luciferase. |

## Background

[PubMed]

Proteins engineered to generate fluorescent or bioluminescent signals are commonly used for the non-invasive study of a variety of biological processes and diseases in animals and humans (1-3). Usually the enhanced green fluorescent proteins, the variant red or yellow fluorescent proteins, or the bioluminescent luciferase proteins are used as reporter molecules because they produce a photo signal upon stimulation that can be tracked for the study of a dynamic biological system or phenomenon under *in vitro* and *in vivo* conditions (4-6). The luciferase family is composed of several different enzymes that catalyze light-producing, oxygen-dependent molecular reactions and have been detected across a diverse group of organisms including bacteria, fungi, insects, and marine animals (7). Among the luciferases, the firefly (*Photinus pyralis*) luciferase (FLuc) has been

[1] National Center for Biotechnology Information, NLM, NIH, Bethesda, MD 20878; Email: micad@ncbi.nlm.nih.gov.

NLM Citation: Chopra A. *Gaussia princeps* luciferase . 2008 Jan 31 [Updated 2008 Feb 28]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

extensively studied; it requires ATP and $Mg^{2+}$ as co-factors to function and oxidizes D-luciferin, its substrate, to release energy in the form of photons (8). The sea pansy (*Renilla reniformis*) is another luciferase (RLuc) that has been extensively investigated; RLuc uses coelenterazine as a substrate and does not require ATP to produce light. However, RLuc has a lower quantum yield compared with FLuc (6% *versus* 88%) and also shows a low enzymatic efficiency (9). With the use of commercially available molecular kits, the humanized forms of FLuc and RLuc (designated as hFLuc and hRLuc, respectively) were combined as dual reporters to image cultured cells *in vivo* because both enzymes used different substrates (10, 11). However, these enzymes have very specific requirements to catalyze reactions under *in vivo* conditions to generate sufficient signal/background ratios for imaging. Tannous et al. decided to explore the possibility of finding a superior luciferase that was more efficient and had a higher quantum yield compared with the FLuc and RLuc enzymes (12).

*Gaussia princeps*, a marine copepod, naturally secretes a luciferase (GLuc) that was cloned, expressed in *Escherichia coli*, and used as a detection agent in a DNA hybridization assay (13). Tannous et al. cloned the humanized version of the GLuc enzyme (hGLuc) into a herpes simplex virus-1 (HSV-1) amplicon under a cytomegalovirus (CMV) immediate early (IE) promoter and evaluated the bioluminescence emitted from DNA-transfected and vector-infected mammalian cells (12). The luciferase was also evaluated for imaging cultured cells *in vivo* after subcutaneous implantation of GLuc-transfected cells into nude mice (12). In another study, GLuc was evaluated for imaging after it was cloned into a lentivirus (LV) vector and transduced into human glioma cells that were implanted into nude mice (14).

## Synthesis

[PubMed]

The cDNA encoding hGLuc, with codons optimized for mammalian cell expression, was amplified by polymerase chain reaction from a commercially available vector with the use of appropriate primers (12). The primers introduced an *EcoR*I site at the 5' end and an *Xho*I site at the 3' end of the cDNA. The polymerase chain reaction product was digested with these enzymes, then gel-purified and cloned into the pHGCX amplicon plasmid described elsewhere (15) under the control of a CMV IE promoter. Because FLuc and RLuc are well studied, hFLuc or hRLuc cDNA, with codons optimized for mammalian cell expression and cloned into pHGCX as described by Shah et al., were used as controls in the study (11). All amplicon constructs were packaged in the HSV-1 virions with the use of a virus-free packaging system and a cell line derived from vero cells (15, 16). The cell growth medium containing the amplicon vector was concentrated and purified through a 25% sucrose cushion by centrifugation for 3 h at 4°C. The amplicon vector titer was determined in a serial dilution assay by fluorescent microscopy for the green fluorescent protein expressed by infected vero cells.

The hGLuc cDNA was also cloned into a LV vector under the control of a CMV promoter to produce LV-GLuc as detailed by Wurdinger et al. (14) For the same study, the hGLuc DNA was also cloned in a LV vector optimized for cyan fluorescent protein separated either by an internal ribosomal entry site or the secreted alkaline phosphatase (SEAP). Another LV construct contained the cDNA sequence for the *mCherry* protein separated by SEAP (the *mCherry* protein is described by Shaner et al. (17)). All of the respective LV constructs were produced by transfection of 293T cells with the LV vector plasmid *pCMVΔR8.91* (the packaging genome plasmid) and the vesicular stomatitis virus envelope glycoprotein plasmid *pVSV-G*. The LV purification was performed by ultracentrifugation of the cell conditioned growth medium and titered as transducing units (t.u.)/ml on 293T cells in the presence of polybrene by counting the cells positive for cyan or yellow fluorescent proteins 72 h after infection (18). The typical titer was reported to be $10^8$ t.u./ml.

## *In Vitro* Studies: Testing in Cells and Tissues

[PubMed]

Mammalian cells containing the HSV-1 amplicon vectors were reported to express the secreted hGLuc and exhibited bioluminescence characteristics that were similar to those of other coelenterazine luciferases (12). The hGLuc generated a >1,000-fold higher bioluminescent signal intensity from live cells in the presence of the growth medium and a >100-fold higher intensity from viable cells alone (in the absence of the growth medium) or cell lysates compared with either the hFLuc or the hRLuc expressed under the same conditions. Compared with hRLuc, the hGLuc had a 200-fold higher signal intensity and exhibited an intensity comparable to hFLuc under standard *in vivo* imaging conditions. The investigators concluded that hGLuc could provide a sensitive means to image cells and their environment in real time after gene delivery because it has a high signal intensity and is a secreted luciferase that is independent of ATP.

In another study, hGLuc was transduced into 293T cells, and its secretion was monitored by determining the conditioned medium luciferase activity (18). When the cells were treated with different drugs that interfere with the protein secretion pathway at various stages, the secretion of hGLuc was decreased up to 90%. To visualize the secretion pathway of cells in real time, the cells were co-transfected with a plasmid expressing FLuc and a plasmid expressing either hGLuc or SEAP. The FLuc activity was measured with luciferin, and it was used to normalize the hGLuc or SEAP activity. The hGLuc or SEAP activity was measured in the cell-free conditioned (growth) medium by the addition of either coelenterazine (for hGLuc) or chloro-5-substituted adamantyl-1,2-dioxetane phosphate (for SEAP). hGLuc proved very sensitive in the detection of the cellular endoplasmic reticulum (ER) stress that is associated with a temporary reduction in protein processing through the secretion pathway. The hGLuc secretion assay was reported to have a >20,000-fold higher sensitivity compared with the SEAP assay, which is a well-established technique used to monitor protein processing and ER stress in mammalian cells. The investigators concluded that the GLuc assay was a rapid,

quantitative, and sensitive technique to monitor the cellular secretion pathway and ER stress that could be used for high-throughput screening to discover drugs useful in the treatment of conditions that induce or are a consequence of cellular ER stress.

Wurdinger et al implanted different amounts of human Gli36 glioma cells transduced with a LV vector encoding GLuc into the flanks of nude mice to generate tumors in the animals (14).Three days after tumor implantation, GLuc activity was measured with a luminometer by adding coelenterazine to the blood obtained from these mice. The luciferase activity in the blood was determined to be linear with respect to the number of cells implanted into the animals. Some GLuc activity was also detected in the urine of the animals, indicating that it was cleared through the kidneys. The investigators also noted that there was little difference between the activity measurements in whole blood and the serum, suggesting that hemoglobin, which normally affects luciferase activity (19), did not interfere with the GLuc activity under these assay conditions. The GLuc half-life was determined to be ~6 days when the blood or serum samples were stored at 4°C. Under in vivo conditions, the half-life of GLuc was reported to be ~20 min (14). In addition, a comparison of the GLuc and SEAP (after implantation of Gli36 cells transfected with the appropriate vectors expressing the respective enzymes into mice; see Synthesis section above for details of the vectors) activities in the blood revealed that the GLuc assay was >1,000-fold more sensitive compared with the SEAP assay because hemoglobin inhibits SEAP activity. The investigators also demonstrated that the GLuc concentration in blood could be used as a quantitative marker for the tumor cells expressing GLuc in vivo (14).

# Animal Studies

## Rodents

[PubMed]

A nude mouse model ($n$ = 4 animals/group) was used to compare the bioluminescence intensity produced by Gli36 cells respectively expressing the control (no luciferase expression), hGLuc, hFLuc, or hRLuc (12). The mice were implanted with the Gli36 cells transfected with the respective vectors, and the tumors were allowed to grow for up to 5 days. To determine the luciferase activity from the different tumors, the mice were injected with the respective substrates (25–200 μg coelenterazine/mouse for hGLuc and hRLuc, and 4 mg D-luciferine/mouse for hFLuc) through two different routes, either through the tail vein or intracardially. Under either route of administration, the bioluminescence signal obtained with GLuc was reported to be 200-fold higher than that from the tumors that expressed hRLuc and comparable to the signal obtained with the tumors that expressed FLuc as measured under identical conditions. No signal was detected in the control tumors.

To evaluate the use of hGLuc for imaging deep tissue, $1 \times 10^6$ Gli36 cells expressing hGLuc were implanted in the right basal ganglia of five nude mice (12). Seven days after

implantation, the bioluminescence signal from the implanted cells was detected from the tumors after an intravenous injection of 100 µg coelenterazine.

### Other Non-Primate Mammals

[PubMed]

No references are currently available.

### Non-Human Primates

[PubMed]

No references are currently available.

## Human Studies

[PubMed]

No references are currently available.

## Supplemental Information

[Disclaimers]

## NIH Support

Parts of the studies presented in this chapter were funded by NCI grants CA69246, CA86355, CA92782, and 1K99CA126839-01.

## References

1. Barrett T., Koyama Y., Hama Y., Ravizzini G., Shin I.S., Jang B.S., Paik C.H., Urano Y., Choyke P.L., Kobayashi H. In vivo diagnosis of epidermal growth factor receptor expression using molecular imaging with a cocktail of optically labeled monoclonal antibodies. Clin Cancer Res. 2007;**13**(22 Pt 1):6639–48. PubMed PMID: 17982120.
2. Peterson J.R., Infanger D.W., Braga V.A., Zhang Y., Sharma R.V., Engelhardt J.F., Davisson R.L. and Longitudinal Non-invasive Monitoring of Transcription Factor Activation in Cardiovascular Regulatory Nuclei Using Bioluminescence Imaging. Physiol Genomics. 2008.
3. Zinn K.R., Chaudhuri T.R., Szafran A.A., O'Quinn D., Weaver C., Dugger K., Lamar D., Kesterson R.A., Wang X., Frank S.J. Noninvasive bioluminescence imaging in small animals. Ilar J. 2008;**49**(1):103–15. PubMed PMID: 18172337.
4. Li Y., Sierra A.M., Ai H.W., Campbell R.E. Identification of Sites Within a Monomeric Red Fluorescent Protein that Tolerate Peptide Insertion and Testing of Corresponding Circular Permutations. Photochem Photobiol. 2008;**84**(1):111–9. PubMed PMID: 18173710.

5. Shimozono S., Miyawaki A. Engineering FRET constructs using CFP and YFP. Methods Cell Biol. 2008;**85**:381–93. PubMed PMID: 18155471.
6. Kolossov V.L., Spring B.Q., Sokolowski A., Conour J.E., Clegg R.M., Kenis P.J., Gaskins H.R. Engineering Redox-Sensitive Linkers for Genetically Encoded FRET-Based Biosensors. Exp Biol Med (Maywood). 2008;**233**(2):238–48. PubMed PMID: 18222979.
7. Weissleder R., Ntziachristos V. Shedding light onto live molecular targets. Nat Med. 2003;**9**(1):123–8. PubMed PMID: 12514725.
8. Lembert N., Idahl L.A. Regulatory effects of ATP and luciferin on firefly luciferase activity. Biochem J. 1995;**305**(Pt 3):929–33. PubMed PMID: 7848294.
9. Matthews J.C., Hori K., Cormier M.J. Purification and properties of Renilla reniformis luciferase. Biochemistry. 1977;**16**(1):85–91. PubMed PMID: 12797.
10. Huang Q., Acha V., Yow R., Schneider E., Sardar D.K., Hornsby P.J. Bioluminescence measurements in mice using a skin window. J Biomed Opt. 2007;**12**(5):054012. PubMed PMID: 17994900.
11. Shah K., Tang Y., Breakefield X., Weissleder R. Real-time imaging of TRAIL-induced apoptosis of glioma tumors in vivo. Oncogene. 2003;**22**(44):6865–72. PubMed PMID: 14534533.
12. Tannous B.A., Kim D.E., Fernandez J.L., Weissleder R., Breakefield X.O. Codon-optimized Gaussia luciferase cDNA for mammalian gene expression in culture and in vivo. Mol Ther. 2005;**11**(3):435–43. PubMed PMID: 15727940.
13. Verhaegen M., Christopoulos T.K. Recombinant Gaussia luciferase. Overexpression, purification, and analytical application of a bioluminescent reporter for DNA hybridization. Anal. Chem. 2002;**74**(17):4378–4385. PubMed PMID: 12236345.
14. Wurdinger T., Badr C., Pike L., de Kleine R., Weissleder R., Breakefield X.O., Tannous B.A. A secreted luciferase for ex vivo monitoring of in vivo processes. Nat Methods. 2008;**5**(2):171–3. PubMed PMID: 18204457.
15. Saeki, Y., X.O. Breakefield, and E.A. Chiocca, eds. Improved HSV-1 amplicon packaging system using ICP27-deleted oversized HSV-1 BAC DNA. Viral Vectors for Gene Therapy, Mathods and Protocols, ed. A. Curtis. 2003, Mashida Humana: Totowa, NJ. 51-60.
16. Aubert M., O'Toole J., Blaho J.A. Induction and prevention of apoptosis in human HEp-2 cells by herpes simplex virus type 1. J Virol. 1999;**73**(12):10359–70. PubMed PMID: 10559354.
17. Shaner N.C., Campbell R.E., Steinbach P.A., Giepmans B.N., Palmer A.E., Tsien R.Y. Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. Nat Biotechnol. 2004;**22**(12):1567–72. PubMed PMID: 15558047.
18. Badr C.E., Hewett J.W., Breakefield X.O., Tannous B.A. A highly sensitive assay for monitoring the secretory pathway and ER stress. PLoS ONE. 2007;**2**(6):e571. PubMed PMID: 17593970.
19. Colin M., Moritz S., Schneider H., Capeau J., Coutelle C., Brahimi-Horn M.C. Haemoglobin interferes with the ex vivo luciferase luminescence assay: consequence

for detection of luciferase reporter gene expression in vivo. Gene Ther. 2000;**7**(15): 1333–6. PubMed PMID: 10918505.