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ORIGINAL ARTICLE

# Dual-reporter Imaging and its Potential Application in Tracking Studies

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**Abstract** By tracking reporter molecules such as green fluorescent protein and luciferase, researchers can determine physiological status and follow processes both in vitro and in vivo. Here, we describe a dual-reporter imaging method, in which a fusion of eGFP and Luc2 is introduced into hosts using lentiviral particles based on HIV-1. The fusion molecule is both fluorescent and bioluminescent, and is therefore ideal as an optical marker in clinical and research applications. We characterized multiple technical indices of the molecule, including sensibility, biocompatibility, lifetime, and others. Lentiviral particles carrying the reporter were strongly infective in endothelial progenitor (EPC) and GL261 glioma cells, as well as in live mice. By transforming Luc2-eGFP into hosts, morphological and quantitative data can be collected not only from tissue specimens but also from live animal models.

**Keywords** Molecular imaging · Small-animal imaging · Dual-reporter imaging · Bioluminescence · Fluorescence

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## Introduction

Molecular imaging encompasses several frequently used methods that allow researchers to track specific molecular markers in vivo, as well as follow physiological processes or determine functional status. These techniques have developed to provide not only morphological data [1–5], but also quantitative or other digital information [6–11]. The ideal modality for in vivo imaging requires a sensitive, highly stable, biologically compatible, and relatively nontoxic molecular tracer [12, 13]. In addition, imaging molecules for cytological applications should have low elimination ratio in living cells [14], and should be minimally affected by cell viability or proliferation [15]. One of the most important types of molecular imaging is optical imaging, which is generally used to visualise cells and animal models [16]. For example, researchers use optical imaging to follow the migration, development, differentiation, and therapeutic effects of stem cell transplants [17, 18].

Optical imaging methods are typically based on fluorescence or bioluminescence [19, 20]. Fluorescent proteins are used in the first approach, usually to study morphological structures. On the other hand, by introducing luciferase gene into cells or tissues, bioluminescence imaging generates not only qualitative but also quantitative data. Luciferase uses oxygen, magnesium, and adenosine triphosphate (ATP) to emit light that can be detected by a charge-coupled device [21]. Since mammalian animals or cells do not express luciferase endogenously, the background is much lower. For the same reason, bioluminescence imaging is advantageous in terms of signal to noise ratio, practicality, and specificity [22, 23].

We have developed a dual-reporter method that exploits both fluorescence from enhanced green fluorescent protein (eGFP) and bioluminescence from luciferase. By integrating

a fusion of Luc2 and eGFP into host chromosomal DNA, bioluminescent and fluorescent markers maybe expressed as required in regions or structures of interest, both in cells or living animals. To demonstrate this technique, we transformed the dual-reporter construct into mouse brains using HIV-1 lentiviral particles, which were prepared from HEK293T cells by a three-plasmid lentiviral system. We evaluated multiple technical properties of the tracer molecule, including sensibility, biocompatibility, and lifetime. For instance, we found that quantifiable optical signals from luciferase [21, 24] are sustained for at least 28 days in the mouse brain. Furthermore, by modifying the lentiviral vector, other genes could potentially be introduced to enable gene function studies in specific tissues or cells in vivo.

## Materials and Methods

### Construction of FUGW-Luc2-eGFP

A 1652-bp fragment of *luc2* was amplified by PCR from the plasmid *p*GL4.17 [25] using primers with sequence *5′-GAC TCT AGA GCC ACC ATG GAA GAT GCC AAA AAC ATT AAG AA-3′* and *5′-CGG GGA TCC TTC ACG GCG ATC TTG CCG CCC TT-3′*. The fragment was then subcloned into a hUbC-driven eGFP lentiviral plasmid, FUGW vector [26] using *Xba*I and *BamH*I. The final construct FUGW-Luc2-eGFP (reporter construct) was verified by sequencing.

### Lentivirus Preparation

We used a three-plasmid system to prepare lentiviral vectors [27–29] from HEK293T cells. Cells were transiently transfected with Lipofectamine™2000 (Invitrogen) containing the reporter construct FUGW-Luc2-eGFP, the packaging plasmid pCMV-deltaR8.2 (Addgene, USA), and the envelope plasmid pCMV-VSV-G (Addgene, USA) in order to produce pseudotyped viral vectors that are secreted into the culture supernatant. The plasmids were transfected at molar ratio 4:3:2. The culture supernatant was harvested 48 h after transfection, and gently mixed with PEG8000 at a final concentration of 20 %. Vectors were then precipitated by centrifugation for 30 min and 4 °C at 1500×*g*, and re-suspended in 25 mM HEPES pH 7.05 containing pre-cooled, sterilized phosphate buffer saline (PBS) pH 7.4. Vectors were re-suspended in 1/100 volume of the original culture supernatant.

### Infective Titre

The tenth echelon dilution method was used to measure infective virus titer [30]. Briefly, HEK293T cells were plated in a 96-well plate at a density of $2\times10^4$ cells/100 μL in each well, and infected with a serial dilution of lentiviral vectors. Culture media were replaced with fresh media 6 h after infection, and the number of fluorescent cells in each well was determined 96 h after infection. Viral titer was calculated based on the highest dilution factor that produced fluorescent cells.

### Infectivity in Cells

Frozen endothelial progenitor cells (EPC) and GL261 glioma cells were thawed and cultured. Cells were then dissociated using 0.25 % (*w/v*) trypsin, and transferred to serum-free media containing 5 mg/mL polybrene (Sigma-Aldrich) as well as lentiviral particles at multiplicity of infection (MOI) 100. Transduction was analysed by immunocytochemistry and flow cytometry 72 h after transfection.

For immunocytochemistry, cells were mounted on slides, fixed with pre-cooled acetone, and incubated for approximately 30 min in PBS containing 3 % bovine serum albumin, 0.1 % gelatine, and 0.1 % Triton X-100. Samples were probed with 1:500 anti-GFP (Life Technologies) in PBS, and then labelled for 60 min at room temperature with 1:2000 FITC-conjugated rabbit anti-chicken IgY (Life Technologies) in PBS. Cells were examined through an upright fluorescence microscope (Carl Zeiss, Jena, Germany). For flow cytometry, approximately $10^7$ cells were suspended in 1 mL culture media and sorted based on eGFP fluorescence. Cells were analyzed with a FACScan instrument (FACSCalibur, BD Biosciences, USA). A total of 5000 events were analysed for each sample.

### Transduction in Mice

Mice were anaesthetized for 3 min with 3.5 % isoflurane in a semi-airtight chamber, and placed in a stereo-taxic frame. During surgery, animals were administered 1 % (*v/v*) isoflurane, 67 % (*v/v*) nitrogen, and 32 % (*v/v*) oxygen at standard atmospheric pressure through a respiratory mask. A small hole was drilled into the skull, 1.5 mm to the lower right of the bregma midpoint. A virus suspension (2 μl) was injected 1.5 mm underneath at 0.4 μL/min. The needle was kept in place for 3 min after injection. After surgery, mice were monitored until consciousness was regained.

### In Vivo Imaging

Mice were anesthetized, and placed in an IVIS Spectrum in vivo preclinical imaging system (Perkin Elmer Corporation, USA) instrument, a bioluminescence imaging system [31]. Each mouse was analysed at days 1, 3, 7, 14, 21, and 28. Images of regions of interest (ROI) were collected 10 min after intraperitoneal injection of D-luciferin, the luciferase substrate, at 3.75 mg per 25 g mouse weight.

### Ethics Statement

Laboratory mice were provided care according to "*The Care and Use of Laboratory Animals*" by the Laboratory Animal Center of Southeast University.

### Analysis of Protein Structures

Protein structures were obtained from the PDB (http://www.rcsb.org/pdb/home/home.do), and PyMol was used for structural analysis and alignment.

## Results and Discussion

### Generation of Lentiviral Vectors Based on HIV-1

To insert two reporter genes in chromosomal DNA, we used a three-plasmid packaging system to produce lentiviral vectors based on HIV-1. The first plasmid, the reporter construct FUGW-Luc2-eGFP (Online Resource 1), contains a fusion of luciferase and eGFP, and was generated by subcloning *luc2* into an empty FUGW plasmid. Clones were verified by restriction enzyme digestion and PCR to contain fragments with nearly identical electrophoretic mobility of about 1652-bp (Fig. 1). Clones were sequenced for further verification.

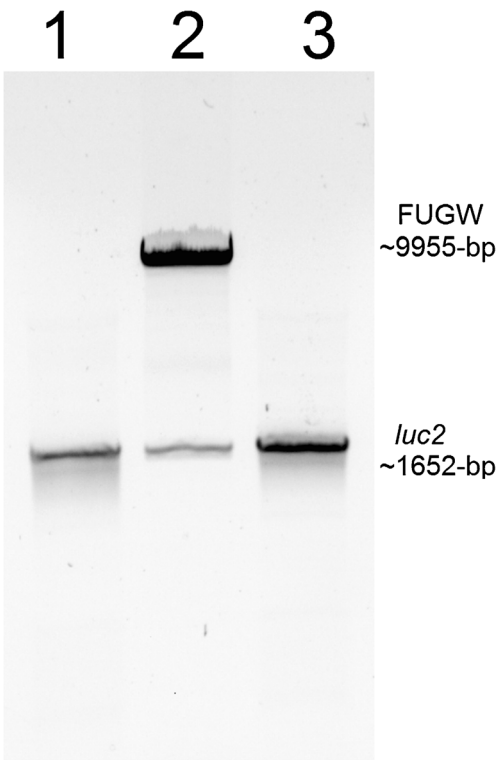


**Fig. 1** Agarose gel electrophoresis of reporter constructs. Lanes 1 and 3, PCR product from *p*GL4.17 (lane 1) and FUGW-Luc2-eGFP (lane 3); lane 2, FUGW-Luc2-eGFP digested with *Xba*I and *BamH*I

FUGW-Luc2-eGFP was then transfected into HEK293T cells, along with the plasmids pCMV-deltaR8.2 and pCMV-VSV-G, to produce lentiviral vector particles, which were secreted into the culture media [32] and harvested 48 h after transfection. The particles were then assayed for infectivity.

### Infectivity Titre

To measure infection titre, we used the simplest available method, in which the viral stock is serially diluted until the number of host cells far exceeds the number of active particles. At this point, every single active particle present would have infected a cell. Thus, HEK293T cells were infected with a serial dilution of lentiviral particles, and the number of resulting fluorescent cells was counted The infection titre of the lentiviral particles was $10^9$ transducing units per mL, based on the number of fluorescent HEK293T cells after infection (Fig. 2).

The infection titre is typically considered to be a measure of the concentration of active lentiviral particles. Nevertheless, it has little real validity, but is only used to calculate MOI [33], which is the ratio of active viral particles to host cells.

### Infectivity in Cells

The infectivity of viral preparations was tested on endothelial progenitor cells (EPC) from human umbilical cord blood [34] and GL261 glioma cells from a murine brain tumour [35]. Both cell lines were infected at MOI 100. Immunocytochemistry indicates that a significant majority of endothelial progenitor and GL261 cells expressed eGFP after infection (Fig. 3). The fraction of fluorescent endothelial progenitor and GL261 cells was 84.8 and 91.69 %, respectively, based on flow cytometry (Fig. 3).

Dual-reporter imaging enables the use of trackable, quantifiable, and biocompatible reporters to investigate differentiation, cell cycling, drug effects, and processes such as synaptogenesis. For example, a potential application is to track endogenous or implanted stem cells. Hence, we tested endothelial progenitor because of their stem cell-like properties.

However, we foresee the dual-reporter system to have wider application in the development of drugs, especially antitumor agents. Therefore, we also tested GL261, a mouse glioma cell line. To assess the antitumor properties of a

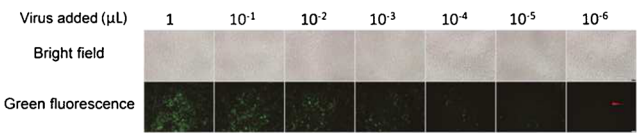


**Fig. 2** Infectivity titre. HEK293T cells were infected with different volumes of lentiviral stock. The minimum volume that produced a fluorescent cell was $10^{-6}$ μL (*red arrow head*). Bright-field images are also shown. Scale bar, 20 μm. Magnification: ×100

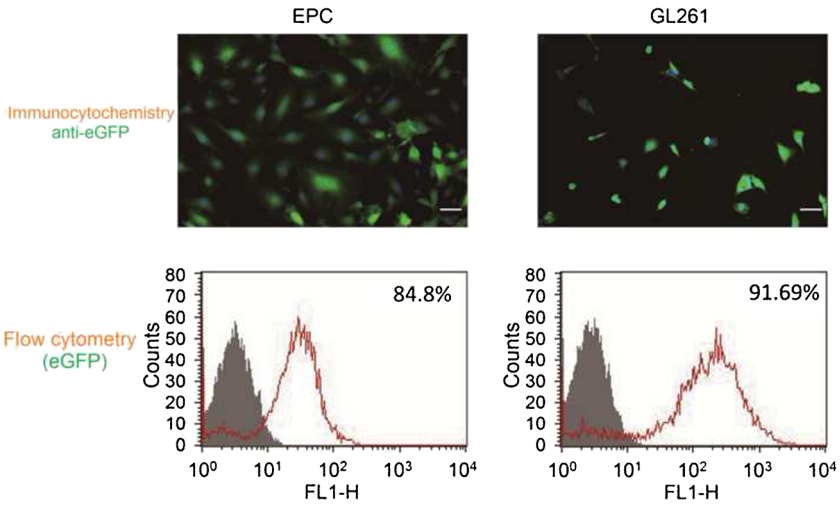


**Fig. 3** Transduced cells. Epithelial progenitor (EPC) and GL261 cells were analysed 72 h after transfection by immunocytochemistry against eGFP, and by flow cytometry based on GFP. Scale bar, 50 μm. Magnification: ×200

compound, researchers may generate tumour models by implanting neoplastic cells or by using genomic mutants. Data are then obtained, traditionally through analysis of the morphological features of a tumour, or through biochemical analysis of samples from the model. In contrast, dual-reporter imaging will allow experimentalists to visualize and quantify the effects of drug intervention in vivo, as well as tumour progression and other carcinogenic processes.

## In Vivo Bioluminescence Imaging

To test if the dual-reporter system is suitable for use in an animal model, we infected mouse brains by orthotopic injection. As a result, *luc2-eGFP* was introduced in part of the cerebral histocyte, although the infection efficiency was unknown. Bioluminescence was evident beginning at day 3. The signal peaked to $10^5$ p/s/cm$^2$/sr at day 14, and remained substantial through day 28 and beyond (Fig. 4). These results indicate that the system can be used in animal models through direct infection of viable tissue. Alternatively, in vivo bioluminescence may be introduced in other ways, such as by implanting cells that are already infected [36]. Notably, the results also imply that D-luciferin crosses the blood-brain barrier, and will probably reach any other region of interest.

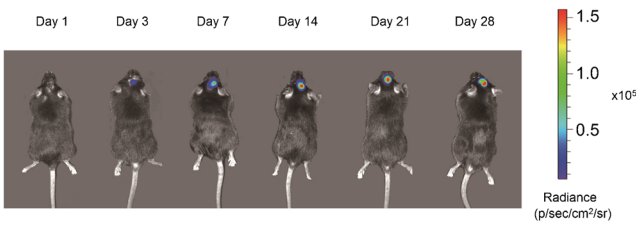


**Fig. 4** Luciferase imaging in vivo. An infected mouse was analysed at days 1, 3, 7, 14, 21, and 28 in an IVIS Spectrum instrument. Bioluminescence intensity is coloured according to the colour scale (×$10^5$ p/s/cm$^2$/sr)

Taken together, the data suggest that the dual-reporter system is a powerful, widely applicable tool.

## Comparison of the Dual-reporter Fusion with eGFP and Luciferase

EGFP fluorescence is easily detected with a flow cytometer or a fluorescence microscope. However, because fluorescence does not penetrate tissues, eGFP is unsuitable for viviperception of animal models, especially when the region of interest is internal. In addition, anti-eGFP may be required to label and enhance eGFP fluorescence. On the other hand, luciferase cannot be directly tracked, because it is not fluorescent and requires luciferin. However, the enzyme emits photons that penetrate tissues, are quantifiable, and enable tracking and visualization of biological processes in vivo. The fused dual reporter, Luc2-eGFP, combines the capabilities of both reporters, and, consequently, acquires significant advantages over each.

A main concern with protein fusions is the possibility of loss of function from either or both components. The beta-barrel structure of eGFP (PDBID: 4EUL) is rigid (Online Resource 2A), and should not be significantly affected by N- or C-terminal fusions. Unfortunately, the available structure for Luc2 (PDBID: 1BA3) does not clearly show the cofactor-binding pocket, which is critical for activity. Therefore, we used the structure of the homolog (PDBID: 2D1R) from the Japanese firefly to locate the cofactor-binding pocket. The two structures are very similar and align well (Online Resource 2B) except at the C-terminus, making the root mean square deviation for global alignment was as high as 1.059. As the cofactor-binding pocket is not at the C-terminus, we concluded that the pocket in Luc2 is formed by the corresponding motifs in the Japanese firefly luciferase (Online Resource 2C). The entrance to this pocket is 29.37 Å from the C-terminus. Hence, fusion of eGFP to the C-terminus should not block the pocket and inhibit enzyme

activity. In summary, the Luc2-eGFP fusion should have fully functional Luc2 and eGFP, although we are unable to provide quantitative evidence.

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**Author Contributions** J. Ding conducted all experiments, integrated data, and edited figures; C. Wang performed the structural analysis and wrote the manuscript; P.C. Li, Z. Zhao, C. Qian, C.X. Wang and Y. Cai provided essential assistance; G.J. Teng directed this study, designed the research and gave key advices.

**Compliance with Ethical Standards** This manuscript has not been published or presented elsewhere in part or in entirety, and is not under consideration by another journal. All study participants provided informed consent, and the study design was approved by the appropriate ethics review boards.

**Conflict of Interest** All authors discussed the results and approved the manuscript. There are no conflicts of interest to declare.

## References

1. Kost B, Spielhofer P, Chua NH (1998) A GFP-mouse talin fusion protein labels plant actin filaments in vivo and visualizes the actin cytoskeleton in growing pollen tubes. Plant J: Cell Mol Biol 16(3): 393–401
2. Dhonukshe P, Laxalt AM, Goedhart J, Gadella TW, Munnik T (2003) Phospholipase d activation correlates with microtubule reorganization in living plant cells. Plant Cell 15(11):2666–2679. doi:10.1105/tpc.014977
3. Mitsiades CS, Mitsiades NS, Bronson RT, Chauhan D, Munshi N, Treon SP, Maxwell CA, Pilarski L, Hideshima T, Hoffman RM, Anderson KC (2003) Fluorescence imaging of multiple myeloma cells in a clinically relevant SCID/NOD in vivo model: biologic and clinical implications. Cancer Res 63(20):6689–6696
4. Kim HS, Cho HR, Choi SH, Woo JS, Moon WK (2010) In vivo imaging of tumor transduced with bimodal lentiviral vector encoding human ferritin and green fluorescent protein on a 1.5 T clinical magnetic resonance scanner. Cancer Res 70(18):7315–7324. doi:10.1158/0008-5472.CAN-10-0241
5. Boumahdi S, Driessens G, Lapouge G, Rorive S, Nassar D, Le Mercier M, Delatte B, Caauwe A, Lenglez S, Nkusi E, Brohee S, Salmon I, Dubois C, del Marmol V, Fuks F, Beck B, Blanpain C (2014) SOX2 controls tumour initiation and cancer stem-cell functions in squamous-cell carcinoma. Nature 511(7508):246–250. doi:10.1038/nature13305
6. Choy G, Choyke P, Libutti SK (2003) Current advances in molecular imaging: noninvasive in vivo bioluminescent and fluorescent optical imaging in cancer research. Mol Imaging 2(4):303–312
7. Kim YJ, Dubey P, Ray P, Gambhir SS, Witte ON (2004) Multimodality imaging of lymphocytic migration using lentiviral-based transduction of a tri-fusion reporter gene. Mol Imaging Biol: MIB: Off Publ Acad Mol Imaging 6(5):331–340. doi:10.1016/j.mibio.2004.06.009
8. Laxman B, Hall DE, Bhojani MS, Hamstra DA, Chenevert TL, Ross BD, Rehemtulla A (2002) Noninvasive real-time imaging of apoptosis. Proc Natl Acad Sci U S A 99(26):16551–16555. doi:10.1073/pnas.252644499
9. Min JJ, Nguyen VH, Kim HJ, Hong Y, Choy HE (2008) Quantitative bioluminescence imaging of tumor-targeting bacteria in living animals. Nat Protoc 3(4):629–636. doi:10.1038/nprot.2008.32
10. Rehemtulla A, Stegman LD, Cardozo SJ, Gupta S, Hall DE, Contag CH, Ross BD (2000) Rapid and quantitative assessment of cancer treatment response using in vivo bioluminescence imaging. Neoplasia 2(6):491–495
11. Wurdinger T, Badr C, Pike L, de Kleine R, Weissleder R, Breakefield XO, Tannous BA (2008) A secreted luciferase for ex vivo monitoring of in vivo processes. Nat Methods 5(2):171–173. doi:10.1038/nmeth.1177
12. Park JH, Gu L, von Maltzahn G, Ruoslahti E, Bhatia SN, Sailor MJ (2009) Biodegradable luminescent porous silicon nanoparticles for in vivo applications. Nat Mater 8(4):331–336. doi:10.1038/nmat2398
13. Vaijayanthimala V, Cheng PY, Yeh SH, Liu KK, Hsiao CH, Chao JI, Chang HC (2012) The long-term stability and biocompatibility of fluorescent nanodiamond as an in vivo contrast agent. Biomaterials 33(31):7794–7802. doi:10.1016/j.biomaterials.2012.06.084
14. Tse JW, Wiebe LI, Noujaim AA (1989) High specific activity [samarium-153] EDTA for imaging of experimental tumor models. J Nucl Med: Off Publ Soc Nucl Med 30(2):202–208
15. Boehm-Sturm P, Mengler L, Wecker S, Hoehn M, Kallur T (2011) In vivo tracking of human neural stem cells with 19F magnetic resonance imaging. PLoS One 6(12), e29040. doi:10.1371/journal.pone.0029040
16. Miranda-Lorenzo I, Dorado J, Lonardo E, Alcala S, Serrano AG, Clausell-Tormos J, Cioffi M, Megias D, Zagorac S, Balic A, Hidalgo M, Erkan M, Kleeff J, Scarpa A, Sainz B Jr, Heeschen C (2014) Intracellular autofluorescence: a biomarker for epithelial cancer stem cells. Nat Methods 11(11):1161–1169. doi:10.1038/nmeth.3112
17. Hara M, Murakami T, Kobayashi E (2008) In vivo bioimaging using photogenic rats: fate of injected bone marrow-derived mesenchymal stromal cells. J Autoimmun 30(3):163–171. doi:10.1016/j.jaut.2007.12.007
18. Song F, Tian M, Zhang H (2014) Molecular imaging in stem cell therapy for spinal cord injury. Biomed Res Int 2014:759514. doi:10.1155/2014/759514
19. Contag CH, Bachmann MH (2002) Advances in in vivo bioluminescence imaging of gene expression. Annu Rev Biomed Eng 4: 235–260. doi:10.1146/annurev.bioeng.4.111901.093336
20. Contag CH, Ross BD (2002) It's not just about anatomy: in vivo bioluminescence imaging as an eyepiece into biology. J Magn Reson Imaging : JMRI 16(4):378–387. doi:10.1002/jmri.10178
21. Hollingshead MG, Bonomi CA, Borgel SD, Carter JP, Shoemaker R, Melillo G, Sausville EA (2004) A potential role for imaging technology in anticancer efficacy evaluations. Eur J Cancer 40(6): 890–898. doi:10.1016/j.ejca.2003.12.018
22. Au JT, Gonzalez L, Chen CH, Serganova I, Fong Y (2012) Bioluminescence imaging serves as a dynamic marker for guiding and assessing thermal treatment of cancer in a preclinical model. Ann Surg Oncol 19(9):3116–3122. doi:10.1245/s10434-012-2313-7
23. Schafer H, Schafer A, Kiderlen AF, Masihi KN, Burger R (1997) A highly sensitive cytotoxicity assay based on the release of reporter enzymes, from stably transfected cell lines. J Immunol Methods 204(1):89–98
24. Lehtinen J, Virta M, Lilius EM (2003) Fluoro-luminometric real-time measurement of bacterial viability and killing. J Microbiol Methods 55(1):173–186

25. Yang XY, Levi D, Ounissi-Benkalha H, Yu XY, Qu HQ, Polychronakos C, Du GH (2012) Screening for novel lead compounds increasing insulin expression in medullary thymic epithelial cells. Eur J Pharmacol 688(1–3):84–89. doi:10.1016/j.ejphar.2012.03.047
26. Chen H, Yao H, Huang L, Shen Q, Jia W, Xue J (2006) Expression of human factor IX gene in murine plasma through lentiviral vector-infected haematopoietic stem cells. Clin Exp Pharmacol Physiol 33(12):1196–1201. doi:10.1111/j.1440-1681.2006.04511.x
27. Poeschla EM, Wong-Staal F, Looney DJ (1998) Efficient transduction of nondividing human cells by feline immunodeficiency virus lentiviral vectors. Nat Med 4(3):354–357
28. Zhao J, Pettigrew GJ, Thomas J, Vandenberg JI, Delriviere L, Bolton EM, Carmichael A, Martin JL, Marber MS, Lever AM (2002) Lentiviral vectors for delivery of genes into neonatal and adult ventricular cardiac myocytes in vitro and in vivo. Basic Res Cardiol 97(5):348–358. doi:10.1007/s00395-002-0360-0
29. Chinnasamy N, Chinnasamy D, Toso JF, Lapointe R, Candotti F, Morgan RA, Hwu P (2000) Efficient gene transfer to human peripheral blood monocyte-derived dendritic cells using human immunodeficiency virus type 1-based lentiviral vectors. Hum Gene Ther 11(13):1901–1909. doi:10.1089/10430340050129512
30. Lee MS, Cohen B, Hand J, Nokes DJ (1999) A simplified and standardized neutralization enzyme immunoassay for the quantification of measles neutralizing antibody. J Virol Methods 78(1–2):209–217
31. Caceres G, Zhu XY, Jiao JA, Zankina R, Aller A, Andreotti P (2003) Imaging of luciferase and GFP-transfected human tumours in nude mice. Lumin: J Biol Chem Lumin 18(4):218–223. doi:10.1002/bio.729
32. Holic N, Seye AK, Majdoul S, Martin S, Merten OW, Galy A, Fenard D (2014) Influence of mildly acidic pH conditions on the production of lentiviral and retroviral vectors. Hum Gene Ther Clin Dev 25(3):178–185. doi:10.1089/humc.2014.027
33. Gilden DH, Wroblewska Z, Kindt V, Warren KG, Wolinsky JS (1978) Varicella-zoster virus infection of human brain cells and ganglion cells in tissue culture. Arch Virol 56(1–2):105–117
34. Eggermann J, Kliche S, Jarmy G, Hoffmann K, Mayr-Beyrle U, Debatin KM, Waltenberger J, Beltinger C (2003) Endothelial progenitor cell culture and differentiation in vitro: a methodological comparison using human umbilical cord blood. Cardiovasc Res 58(2):478–486
35. Saris SC, Spiess P, Lieberman DM, Lin S, Walbridge S, Oldfield EH (1992) Treatment of murine primary brain tumors with systemic interleukin-2 and tumor-infiltrating lymphocytes. J Neurosurg 76(3):513–519. doi:10.3171/jns.1992.76.3.0513
36. Clark AJ, Safaee M, Oh T, Ivan ME, Parimi V, Hashizume R, Ozawa T, James CD, Bloch O, Parsa AT (2014) Stable luciferase expression does not alter immunologic or in vivo growth properties of GL261 murine glioma cells. J Transl Med 12(1):345. doi:10.1186/s12967-014-0345-4