Company Overview

Targeting Systems (www.targetingsystems.net)

Dr. Rampyari Walia

1453 North Cuyamaca Street
El Cajon, CA 92020
Phone: 619-562-1518
Fax: 619-562-1326

Products/custom services in optical imaging/ cancer research

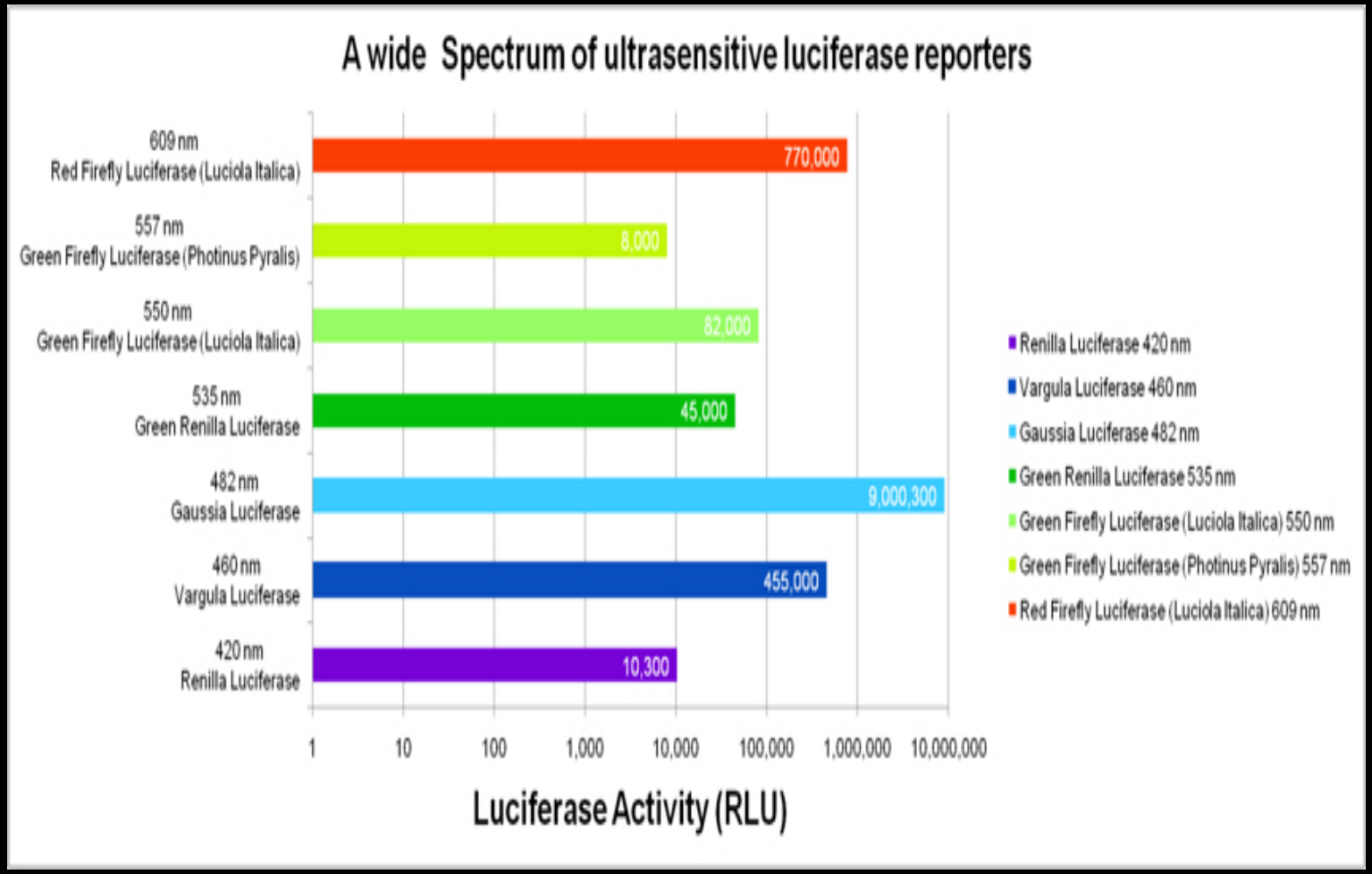
- LentiGlo™ – ready to use lentiviruses co-expressing a novel luciferase along with the flourescent protein
- Bioluminecent tumor cell lines
- Imaging substrates for firefly, Renilla, Gaussia and Cypridina luciferases
- Reagents for quantitative measurement of secreted Gaussia luciferase and Cypridina luciferase activities in the blood
- Our lentiviruses are SIN vectors (ie self inactivating so once they integrate into the cell they no longer produce live virus so are much safer to use)

1. CUSTOM LENTIVIRAL VECTORS (LENTIPLASMIDS OR PACKAGED LENTIVIRUS) EXPRESSING GENE OF INTEREST OR BIOLUMINESCENT REORTERS UNDER CUSTOM PROMOTERS OR RESPONSE ELEMENTS

2. CUSTOM BIOLUMINESCENT CELL LINES FOR TUMOR IMAGING OR STUIDEIS OF GENE EXPRESSION

targetingsystems

Luciferase activities & emission max of different luciferase reporters



Substrate specificities of different luciferases

LUCIFERASE	SUBSTRATE
Gaussia luciferase	**Coelenterazine**
Renilla luciferase	**Coelenterazine**
Red Luciola luciferase	**Firefly luciferin**
Cypridina luciferase	**Cypridina luciferin (Vargulin)**

Options for bioluminescent cell lines

REPORTER OPTIONS:
Cell lines express luciferase alone under control of the UBC promoter or co-express both luciferase and a fluorescent protein under control of the UBC promoter

Luciferase options
Red-emitting firefly luciferase

Green-emitting 40X brighter Renilla luciferase (improved stability and green shifted luminescence emission max 527 nm)

Secreted Gaussia luciferase (Gaussia luciferase emits at 480 nm but very useful to quantitate cell survival by measuring Gluc activity in the blood

Secreted Cypridina luciferase- secreted reporter

Options for fluorescent proteins to be co-expressed with luciferase
GFP, mScarlet, mVermilion (the latter two ate very bright monomeric red fluorescent proteins 2-3 fold brighter than mCherry
IRFP (infra red-emitting fluorescent protein , emission max 713 nm)

List of lentiviral vectors and products

Lentiplasmids expressing different bioluminescent reporter/ Many available as Ready-to use lentiviruses for Bioluminescent imaging/Cell tracking

Lentiplasmids expressing a very bright red-emitting firefly luciferase (Luciola Italica)
pLenti-UBC-RedFluc-T2A-Puro (other options Neo, Hygro resistance); Cat #pLP-30
pLenti-UBC-RedFluc-T2A-EGFP; Cat # pLP-31
pLenti-UBC-redFluc-t2A-Tdtomato Cat# pLPF-CUST-tdtomato
Note Above lentiplasmids (in yellow) are also available as ready to use lentiviruses from Perkin Elmer under the name fo Redfifect. These lentiviruses have been used by Perkin Elmer to engineer some 50 tumor cell lines (Bioware Brite)
http://www.perkinelmer.com/lab-solutions/resources/docs/TCH_Technical_data_sheet_for_CLS960002.pdf
file:///C:/Users/sunil/Desktop/BRO_In_Vivo_Agents_Bioware_Brite.pdf
Advantages: Much brightetr than Luc2 (cell lines are 10-15 fold brighte tthan Luc2 lines, Emission max 617 nm makes it better suited for in vivo imaging)

Dual luciferase expressing lentivirus--Lenti-CMV-Gluc-T2A-RedFluc-T2A-Puro ; Cat# pLP-42
Advantages: Quantitation of cell survival by measuring secreted Gluc, deep tissue imaging using the RedLFuc reporter

Gaussia luciferase-expressing lentiplasmids (also available as lentiviruses)
Lenti-CMV-Gluc(stable)-T2A-EGFP; Cat # pLP-36
pLenti-NfkB-Gluc-stable-T2A-EGFP; Cat# pLP-37
pLenti-NfkB-Gluc-stable-T2A-EGFP-Ef1-Puro ; Cat# pLP38
pLenti-CMV-GLuc-T2A-puro; Cat #pLP-39
Gluc is secreted so enables quantitation of cell survival in vivo by a blood assay
Co-expression of EGFP or mScarlet useful in stem cell studies (examination of cell morphology(evaluate differentiation) and cellular localization in vivo
Advantages: Gaussia luciferase is the brightest known luciferase 1000X brighter than firefly and renilla luciferase

BRET SENSORS FOR IMPROVED IMAGING
Lumi-Scarlet-1 (Lenti-UBC-mScarlet-Linker-GrFluc; Cat# pLP-50
Very bright Red-shifted firefly luciferase (most of the emissions are >620 nm
Much brighter than Luc2 and the red-emitting Luciola luciferase)

Green Renilla luciferase-expressing lentiplasmids (Green Renilla luciferase mutant is more stable and approx. 30X brighter than native Renilla luciferase)
Lenti-UBC-GrRenLuc-T2A-Puro; cat # pLP-60
Lenti-UBC-grRenLuc-T2A-Tdtomato; Cat # LPR CUST tdtomato

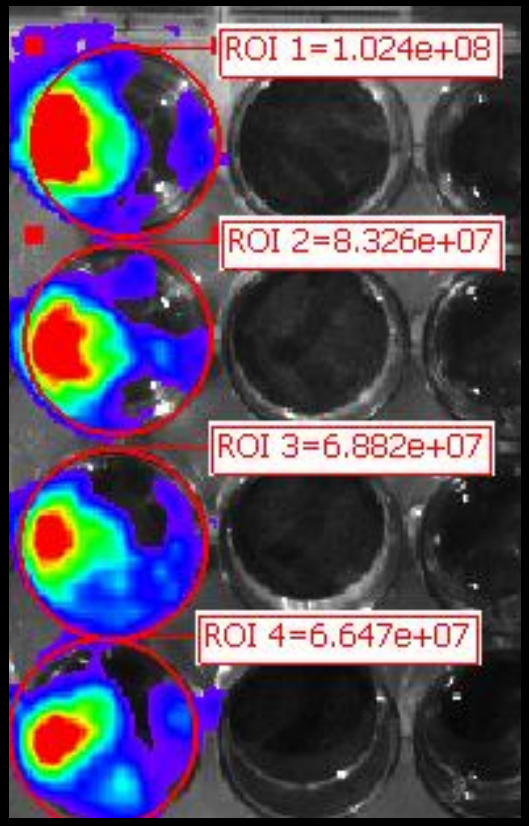
Why LentiGlo™?

- LentiGlo™ vectors contain a bright red-emitting firefly luciferase (emission max 617 nm) or a Green-emitting Renilla luciferase (emission max 527 nm, 35 X brighter than Promega's renilla luciferase) enable improved imaging of deep seated tissues and multiplexing or Gaussia luciferase which is 1000 times brighter than firefly and Renilla luciferases and is a secreted reporter so clel survival in ivivo can also be monitored by mesaruign Gaussia luciferase activity in the blood
- Availability of lentiviruses co-expressing luciferases with GFP , tdtomato or an IRFP (emits at 713 nm) enables visualization of cells by fluorescence ans use of FACs to quickly isolate high expressing stably transfected cells stem cells in the same animal . Many multimodal imaging optiosn
- Lentiviruses c0-expressing secreted Gaussia luciferase (the brightest known luciferase (emission max 480 nm) and fluorescent proteins offer the advantage of measuring tumor growth or survival by BLI and well as measurement of luciferase acitivyt in the blood
- Lentivirus vectors capable of co-expressing red-emitting firefly luciferase and secreted Gaussia luciferase enable visualization of tumor location by BLI as well as quantitative assessment of cell survival/growth by measuring Gaussia luciferase activity in the blood/urine
- Lentiglo vectos expressing both luciferase and GFP/mScarlet) offer advantages for studying stem cell differentiation in viro and in vivo. Data for both applications is available

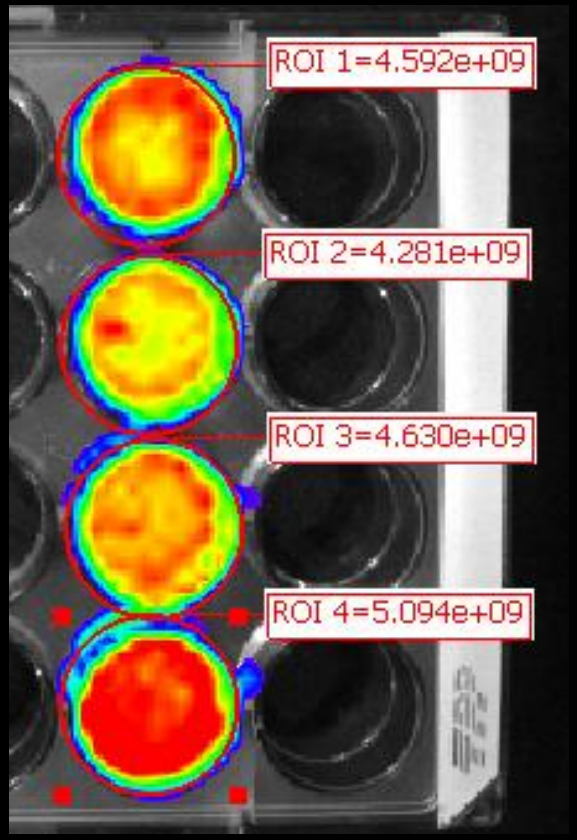
targetingsystems

Imaging of cultured cells expressing RedFluc (left panel) and Gaussia luciferase (right panel)

Luciferin

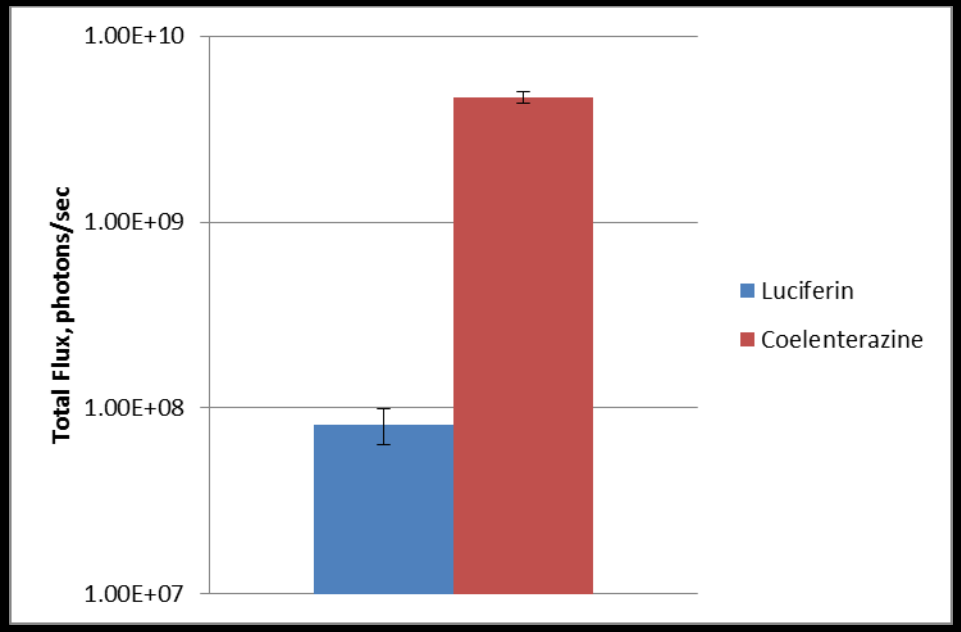


Coelenterazine

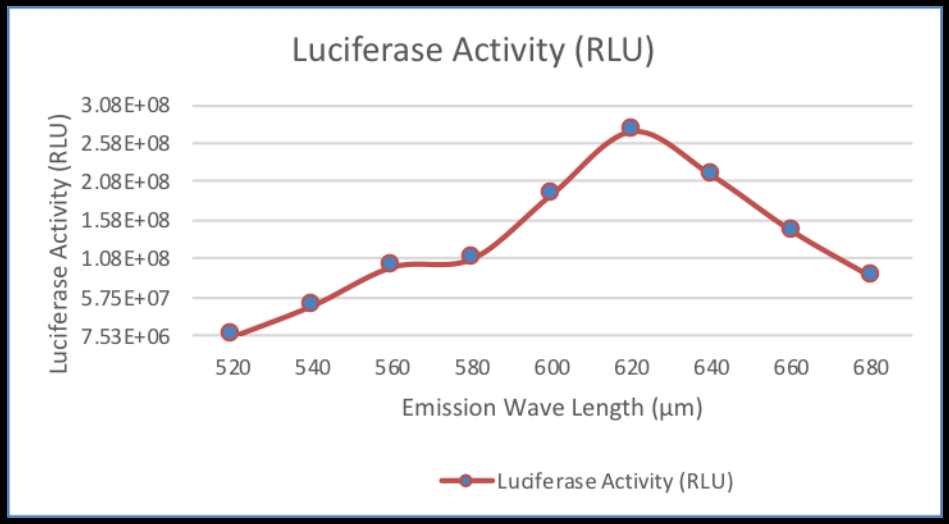


targetingsystems

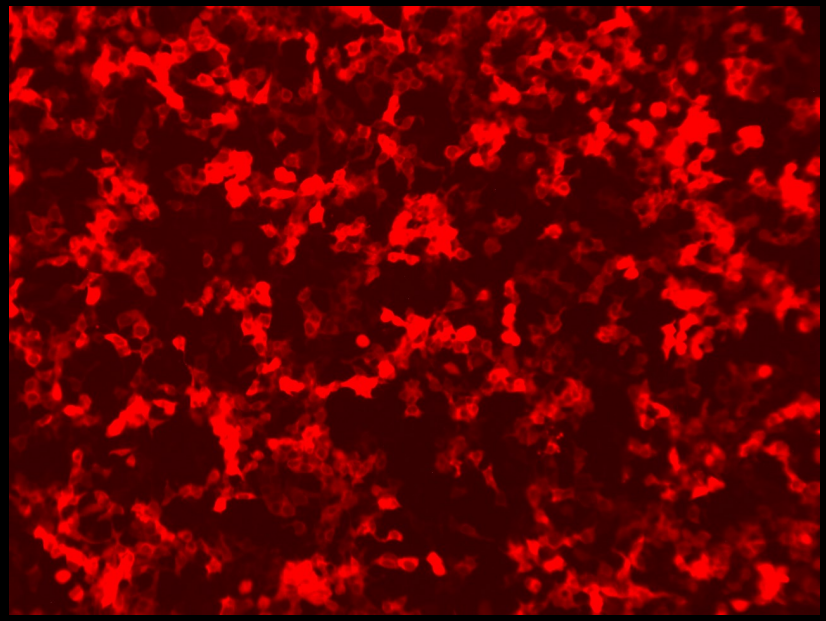
Luciferase activity in cultured cells expressing RedFluc (left panel) and Gaussia luciferase (right panel)



BRET sensors for improved imaging



Emission spectra of luminescence (firefly luciferase acitivty) in cells transfected with the Lumi-Scarlet BRET sensor. Because of BRET the emission of the luciferase partner is red-shifted making it attractive for imaging applications



Fluorescence in HEK-293 cells cells transduced with the lentiviral (plasmid) BRET sensor co-expressing mutant luciferase (green emitting (emission max 560 nm) fused to mScarlet (emission max 590 nm , three times brighter than mCherry)

Applications/Advantages of BRET sensors

- Highly sensitive optical imaging: from single-cell analysis to in vivo whole-body bioluminescence imaging
- Available as a lentiplasmid or a ready-to-use lentivirus
- Strong bioluminescence and fluorescence offers advantage for studying drug - responsiveness or regulation of gene expression. One can monitor gene expression in live cells using fluorescence and assay for luciferase activity at maximal response
- Red-shifted luciferases offers advantages for deep tissue imaging

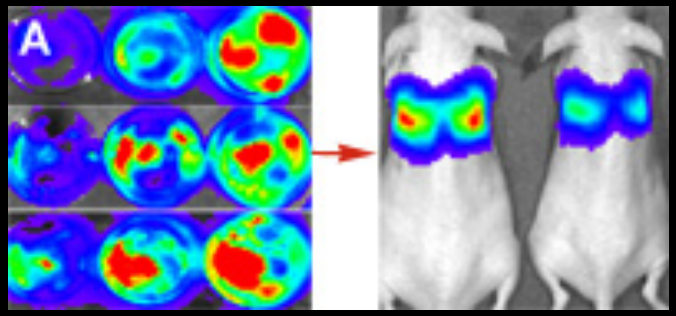
targetingsystems

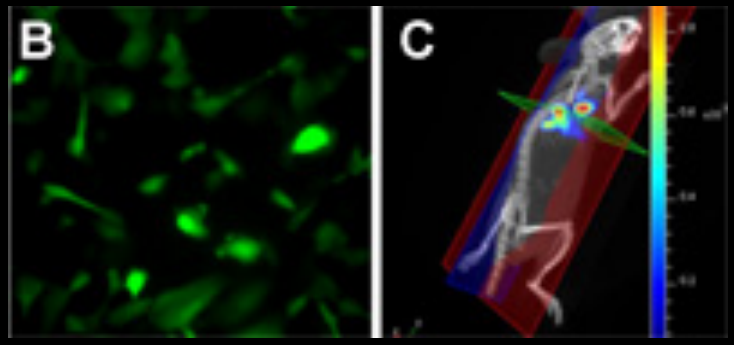
Custom Bioluminescent cell lines constituively expressing Red-emitting firefly luciferase or Green-emitting Renilla luciferase or Gaussia luciferase (single or dual reporter lines)

CELL LINES

- 4T1
- MCF7
- B16F10
- PC3M
- PC3
- LNCaP
- HepG2
- NCI-H460
- U87MG
- GL261
- HT1080
- MDAMB231
- BxPC3
- HT29
- HCT116
- K562
- Colo205
- LL/2
- SKOV3
- A549
- EL4
- MOLT4
- BT474
- CT.26
- ST941C

targetingsystems

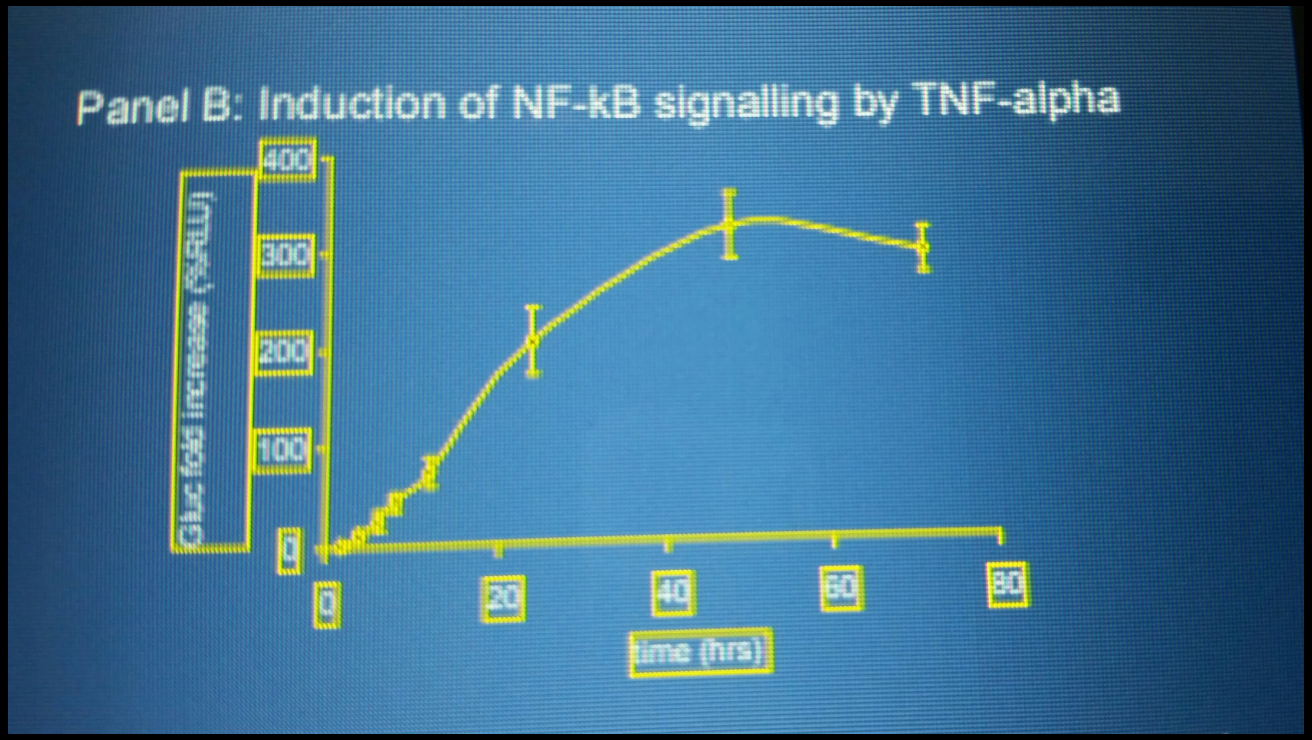




Images above show cells transduced with entiviral particles.co-expressing RedFluc and GFP under control of the UBC promoter (A) *In vitro* luciferase and *in vivo*imaging of PC-3 cells in the lungs. (B) GFP expression and (C) 3D reconstruction of luciferase expressing A549 lung tumors.

targetingsystems

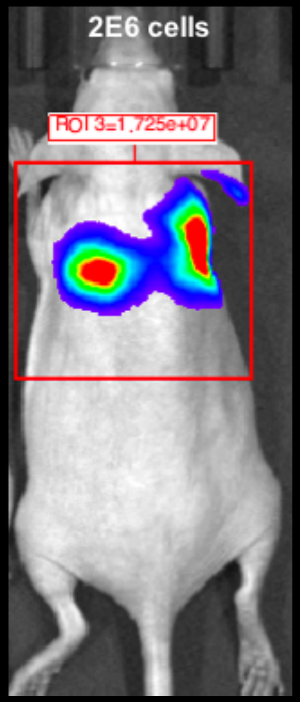
Induction of NF-KB driven Gaussia luciferase in HEK-293 cells by TNF-alpha

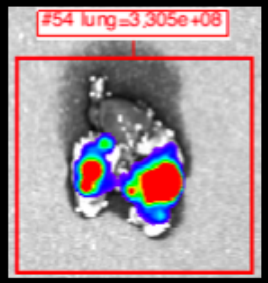


targetingsystems

Data courtesy of Dr Nancy Dumont, Broad iInstitute NCIH2009 cell line transduced with a lentivirus expressing red-emitting firefly luciferase and GFP was imaged with injectable luciferin from Targetign Systems 83 days after post iv innoculation

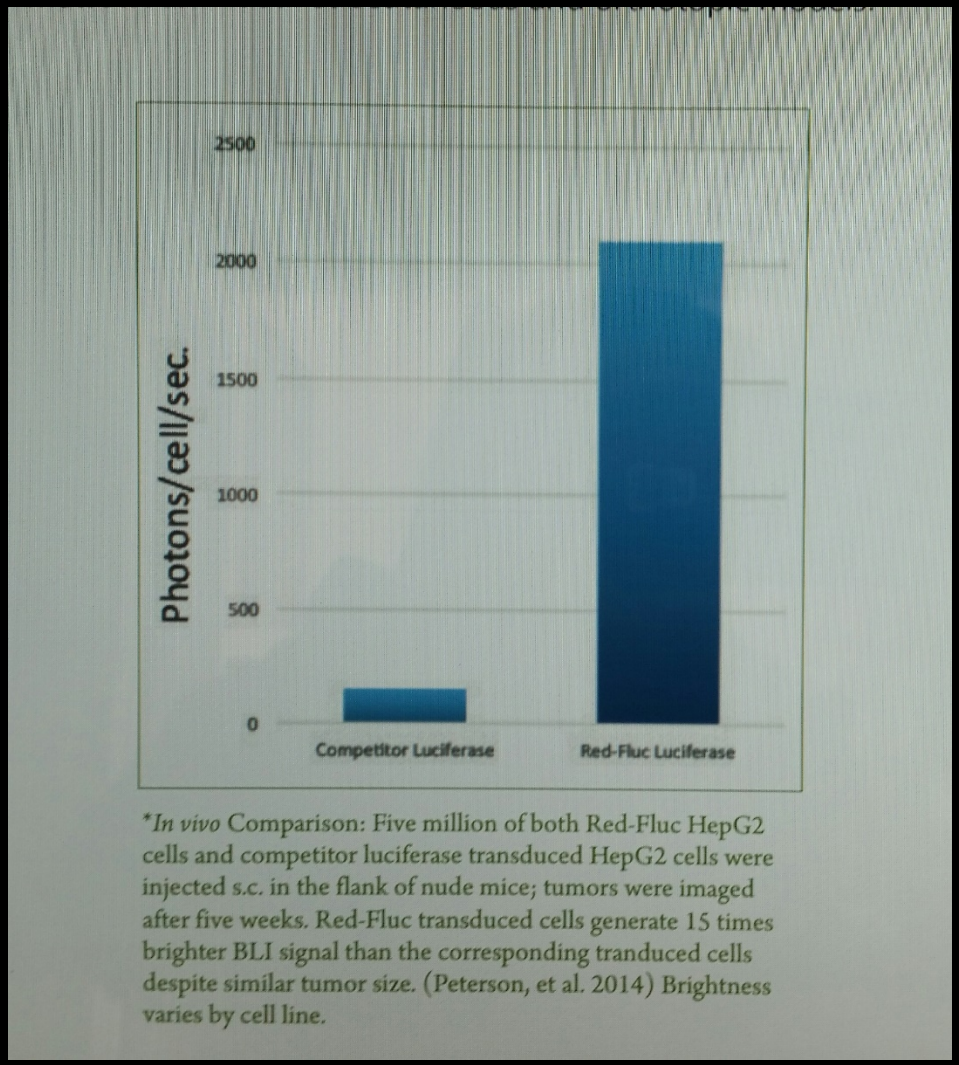
NCI-H2009-rfLuc Mouse #54, 83 days post i.v. Inoculation





targetingsystems

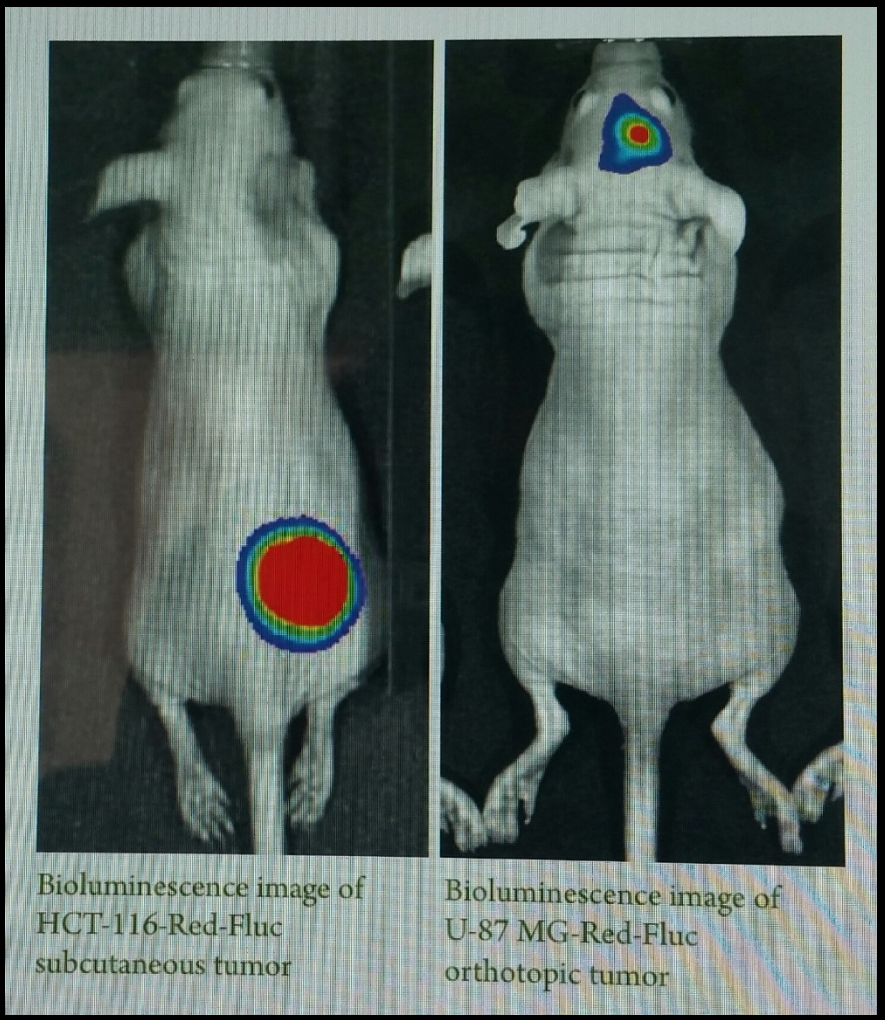
Comparision of RedFluc from Targetign Sysems with Promega Luc2 (their brightest firefly luciferase reporter Data from Perkin Elmer)



**In vivo* Comparison: Five million of both Red-Fluc HepG2 cells and competitor luciferase transduced HepG2 cells were injected s.c. in the flank of nude mice; tumors were imaged after five weeks. Red-Fluc transduced cells generate 15 times brighter BLI signal than the corresponding tranduced cells despite similar tumor size. (Peterson, et al. 2014) Brightness varies by cell line.

targetingsystems

Data on RedFLuc imaging in subcutaneous and orhtotopic tumor models (data from Perkin Elmer)



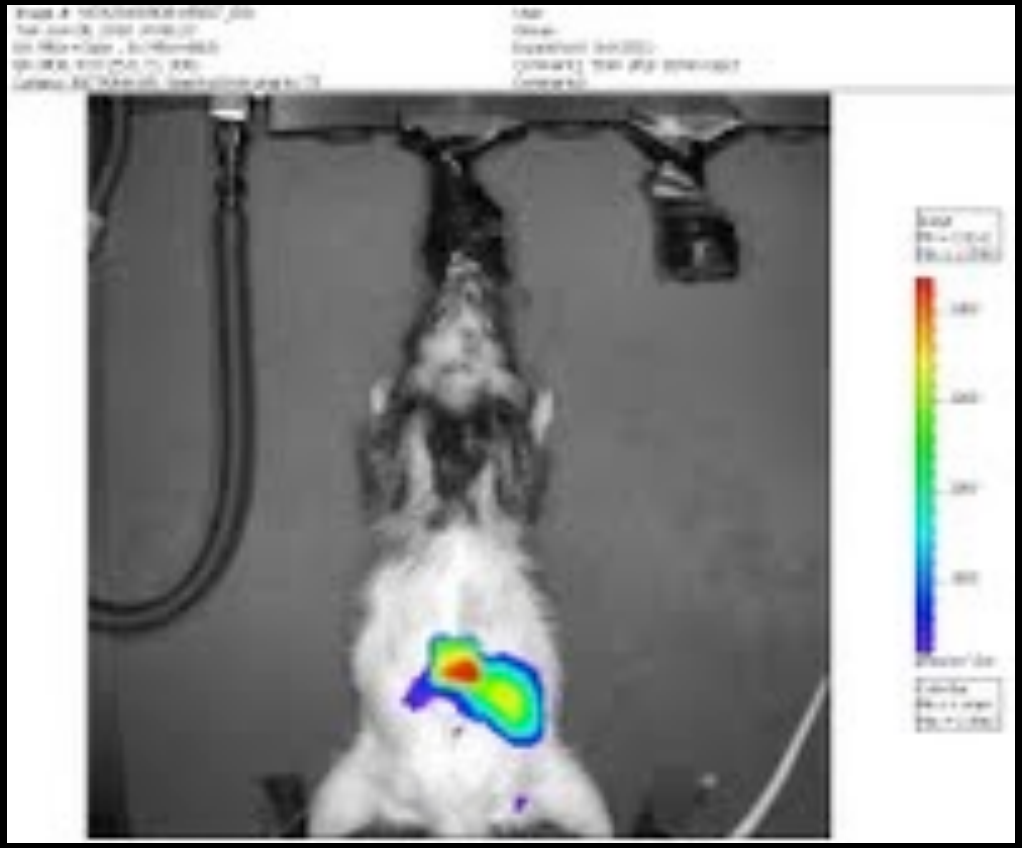
Bioluminescence image of HCT-116-Red-Fluc subcutaneous tumor

Bioluminescence image of U-87 MG-Red-Fluc orthotopic tumor

The optimized Red-Fluc luciferase enablThe optimized Red-Fluc luciferase enables more sensitive in vivo optical imaging with less tissue attenuation so you can detect tumor development earlier, and monitor tumor growth and metastases in both subcutaneous and orthotopic models. es more sensitive in vivo optical imaging with less tissue attenuation so you can detect tumor development earlier, and monitor tumor growth and metastases in both subcutaneous and orthotopic models.

targetingsystems

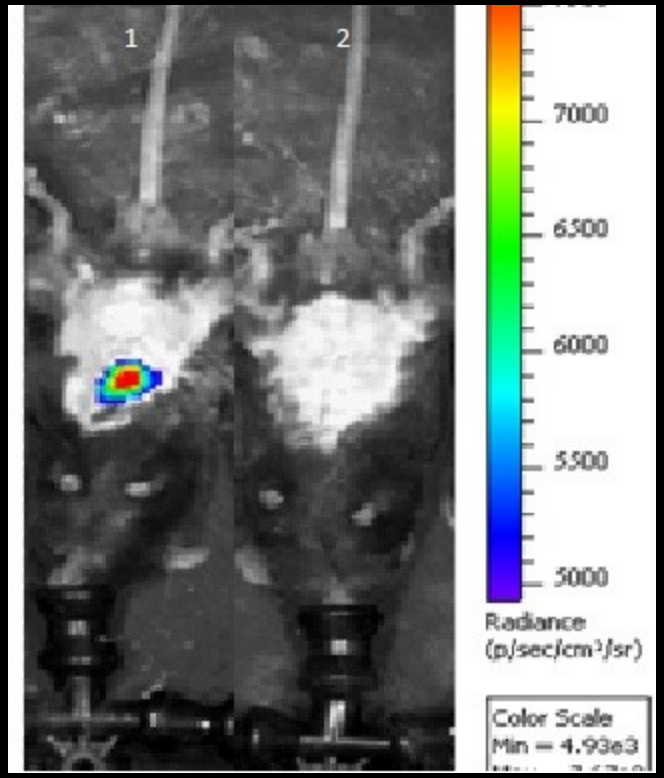
Use of LentiGlo™ (expressing red firefly luciferase) for imaging human stem cells



One million such cells expressing a different luciferase, the red Italica (firefly) luciferase were implanted into the transverse processes in nude mice. After 2 weeks, mice were injected with luciferin intraperitoneally and imaged using a CCD camera. Even though only 500,000 stem cells were implanted a robust signal was observed

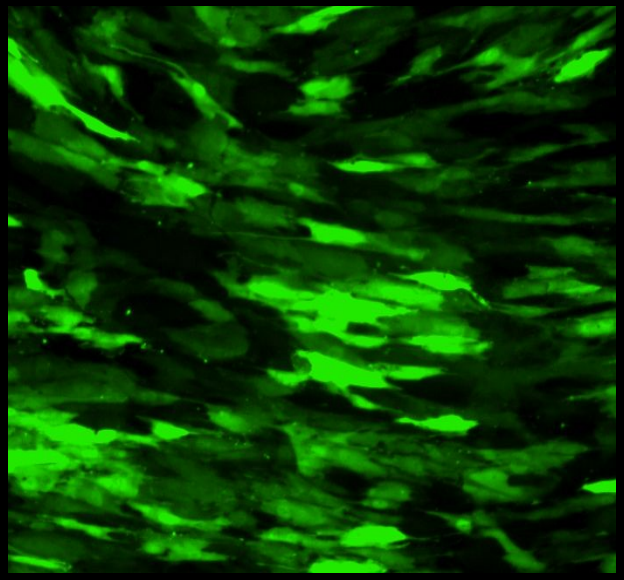
Data courtesy of Lea Kanima and Dr H . Bae, Los Angeles Spine Institute, CA

Detection of intrahepatic implanted liver stem cells



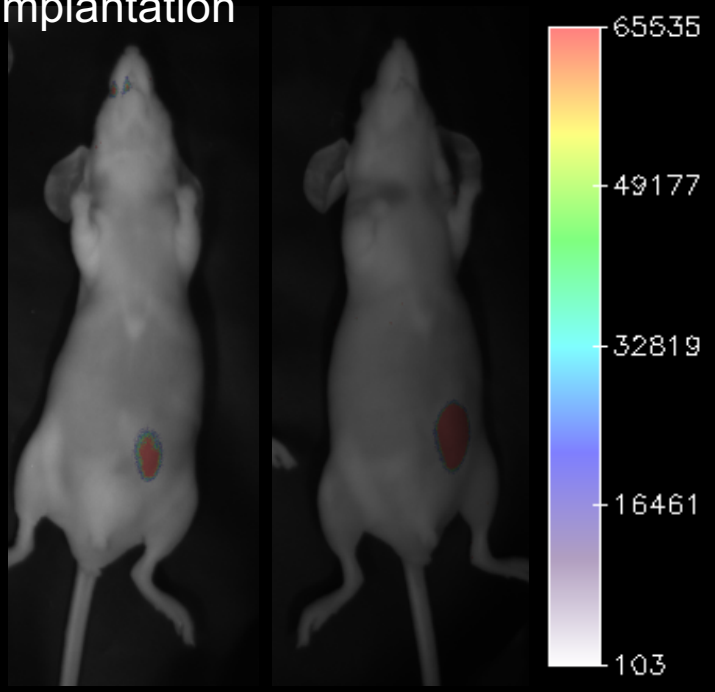
Intrahepatic luciferase luminescence detected by real-time imaging (Xenogen IVIS® Spectrum Bioluminescence). **(1)** Strong luminescence signals are noted on the right upper abdomen of a mouse treated with magnet 14 days post intrahepatic injection of mEP cells labeled with UBC-RedFluc-tdtomato. **(2)** No signal seen in negative control. **Data courtesy of Dr Jeffrey Fair's lab, Cedar Sinai Hospital, Los Angeles, CA.**

Gaussia luciferase as an in vivo reporter to monitor cell survival or gene expression in vivo



1 day Post-implantation

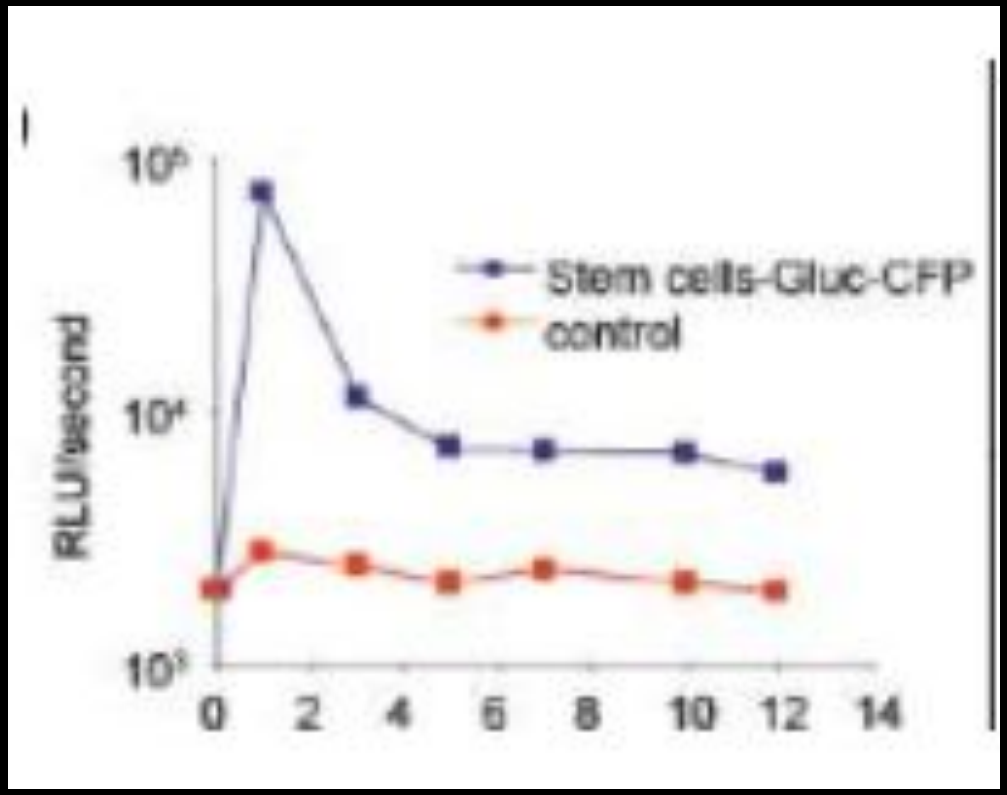
4 days Pos-implantation



Data using primary Ad HMSCs transduced with LentiGlo vector expressing Gluc- GFP and subcutaneously implanted into mice

targetingsystems

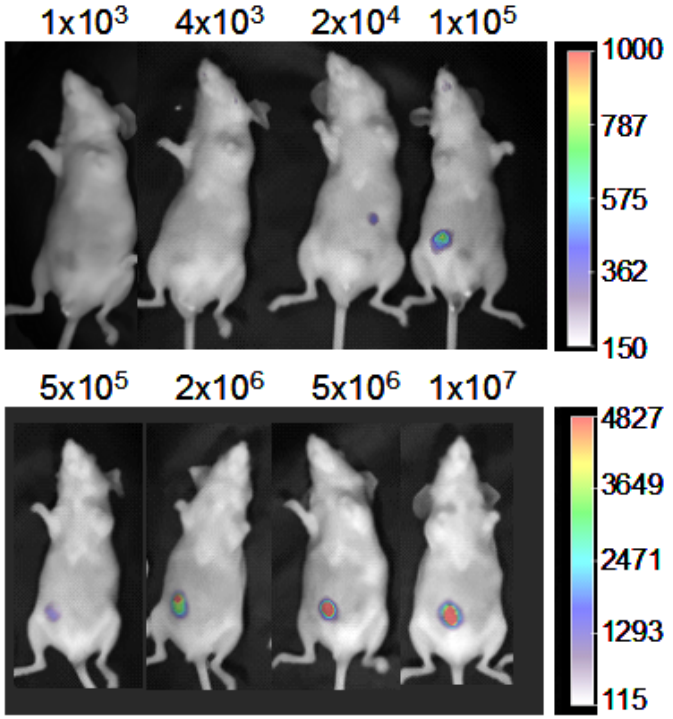
Non-invasive, quantitative measurement of stem cell growth in vivo by measurement of Gluc activity in blood



One millions stem cells expressing Gluc and GFP or PBS control were injected intravenously in nude mice. Prior to injection, the Gluc activity was monitored. The Gluc level in blood indicated that a significant number of cells survived the injection and did not proliferate

targetingsystems

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



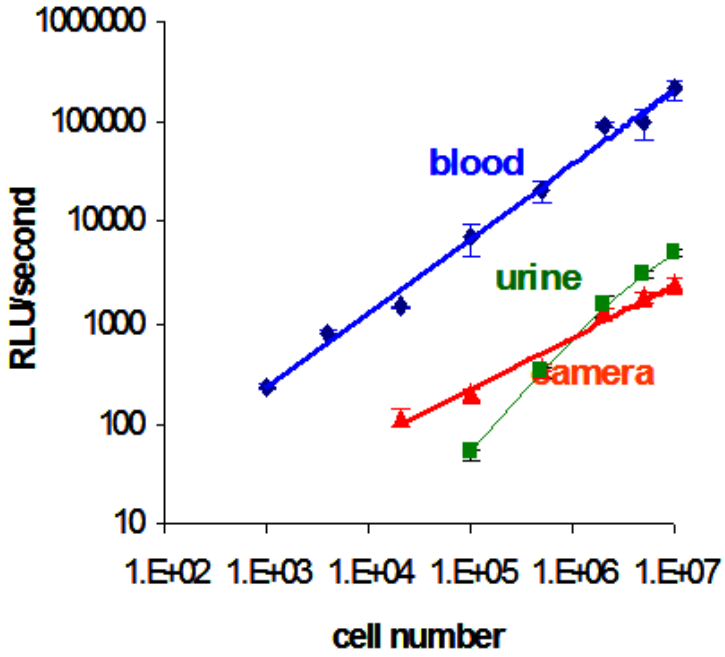
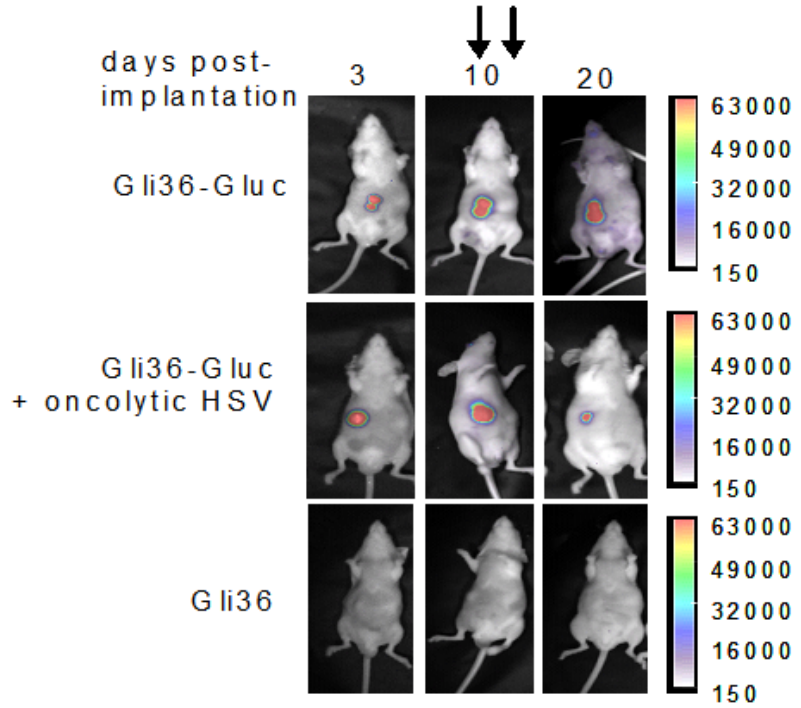


Figure 2. (**Left**) Different numbers of Gli36 human glioma cells expressing-Gluc (Gli36-Gluc) were implanted subcutaneously in mice and 3 days later, mice were injected i.v. with coelenterazine (4 mg/kg body weight) and imaged with CCD camera. (**right**) Total relative light units (RLU) per second was 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)**

Applications of LentiGlo™ technology in tumor imaging

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



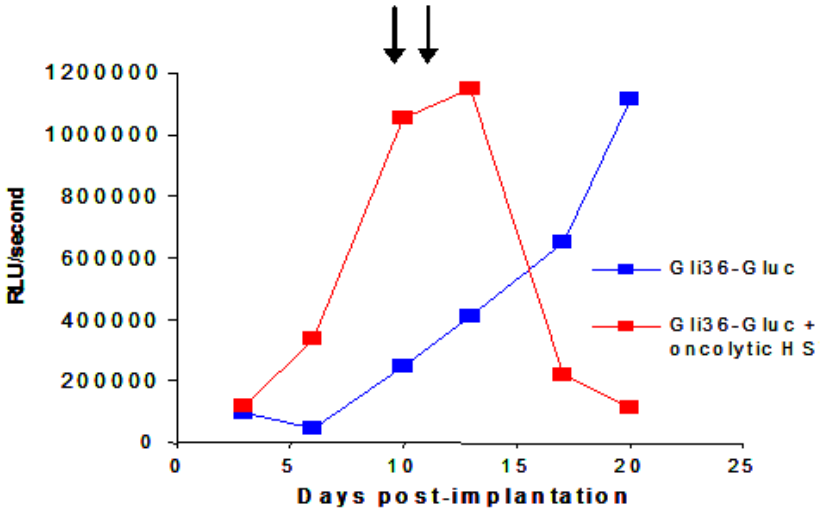


Figure 3. Mice were implanted with one million Gli36-Gluc cells subcutaneously and tumor growth was monitored by both *in vivo* bioluminescence imaging (left) and the Gluc blood assay (right). At day 10 and 13 post-implantation, one set of mice was injected intra-tumorally (arrows) with an oncolytic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

targetingsystems

Directed Protein Delivery

An important tool in stem cell research and therapeutics

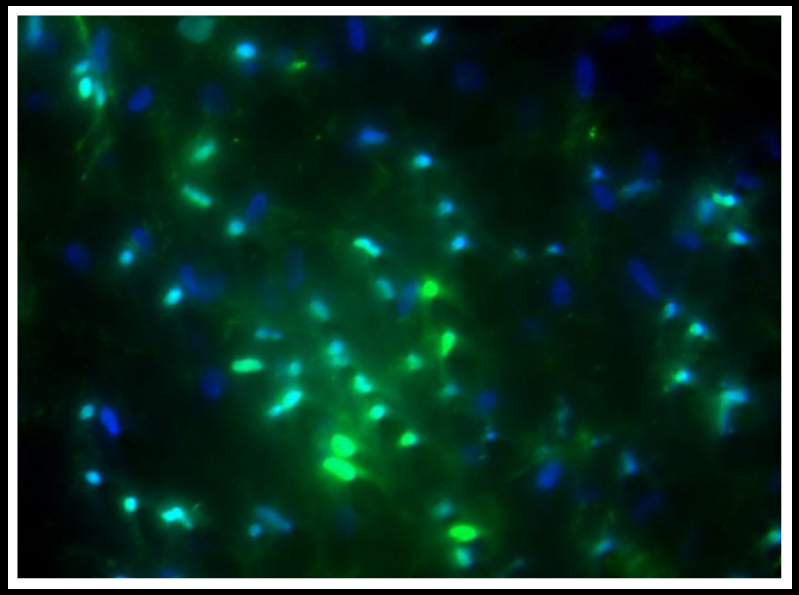
Applications:

- Delivery of transcription factors to mediate differentiation of adult stem cells into specific lineages
- Expansion of stem cells
- Delivery of bioluminescent (chemiluminescent proteins) to track implanted stem cells

Efficient protein delivery into adult human stem cells

Profect™

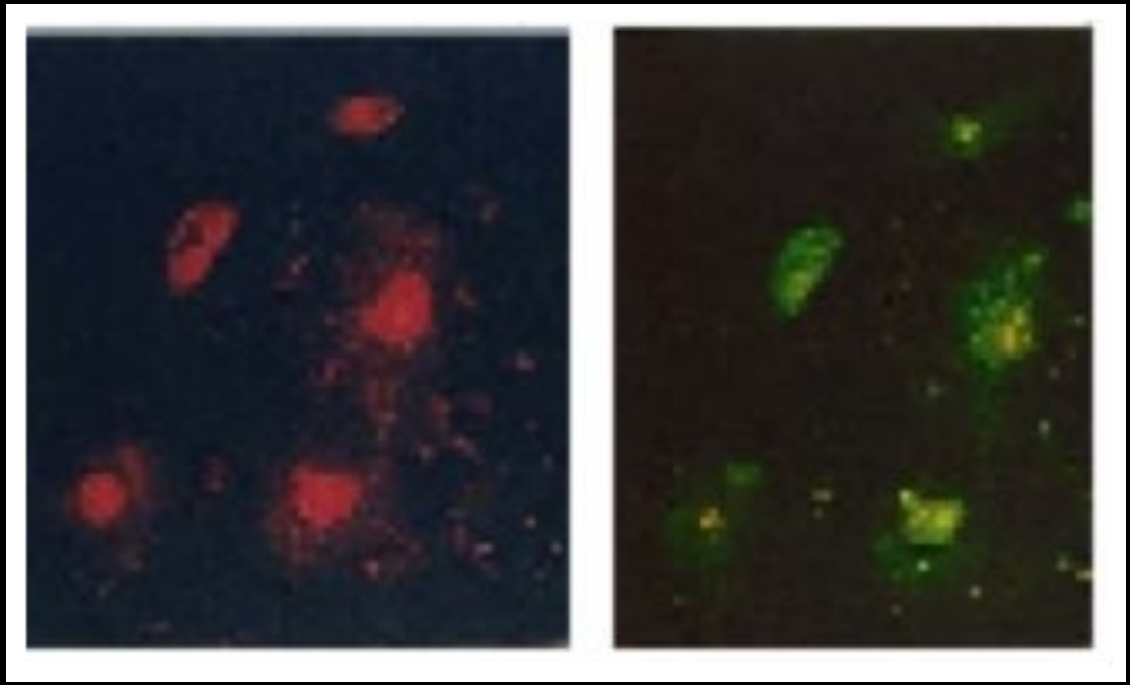
Optimized for protein delivery into stem cells



Mesenchymal Stem Cells transfected with Pluriport showing Histone and Dapi imaged at 20X magnification

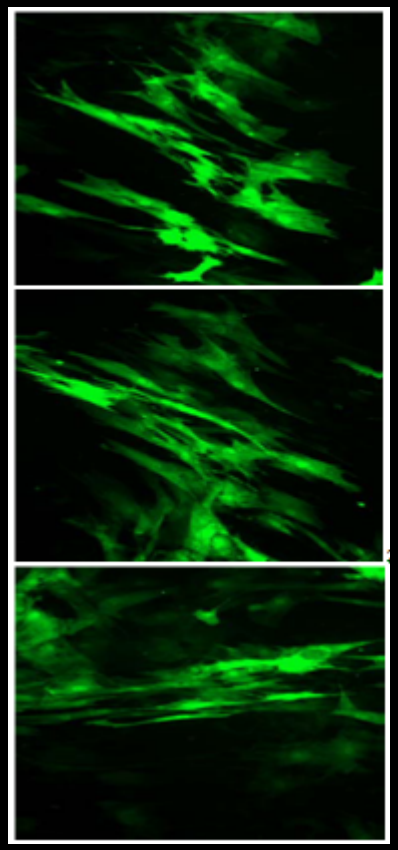
Antibody Delivery

Antibody delivery into the nucleus of Cos-7 cells using Profect P-2

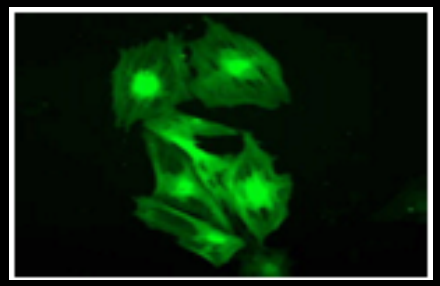


Cos-7 cells were transfected with Alexa 594 conjugated IgG and Alexa 488 conjugated histone using the Profect P-2 reagent.

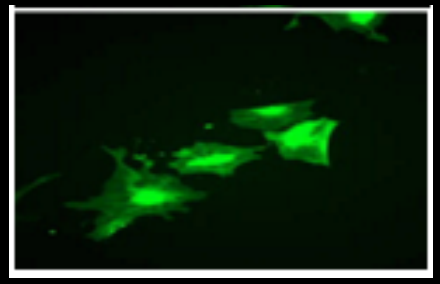
LentiGlo™ Stem Cells



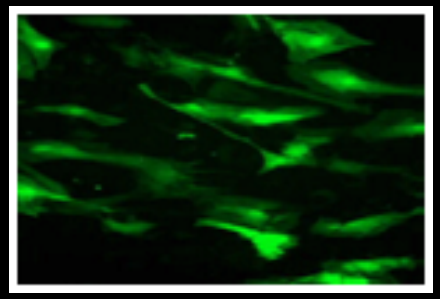
Myogenic pics of pCMV-Gluc-GFP transduced adipose derivered mesenchymal stem cells treated with myogenic treatment



Cardiomyocyte-like pics of pCMV-Gluc-GFP transduced adipose derivered mesenchymal stem cells treated with myogenic treatment



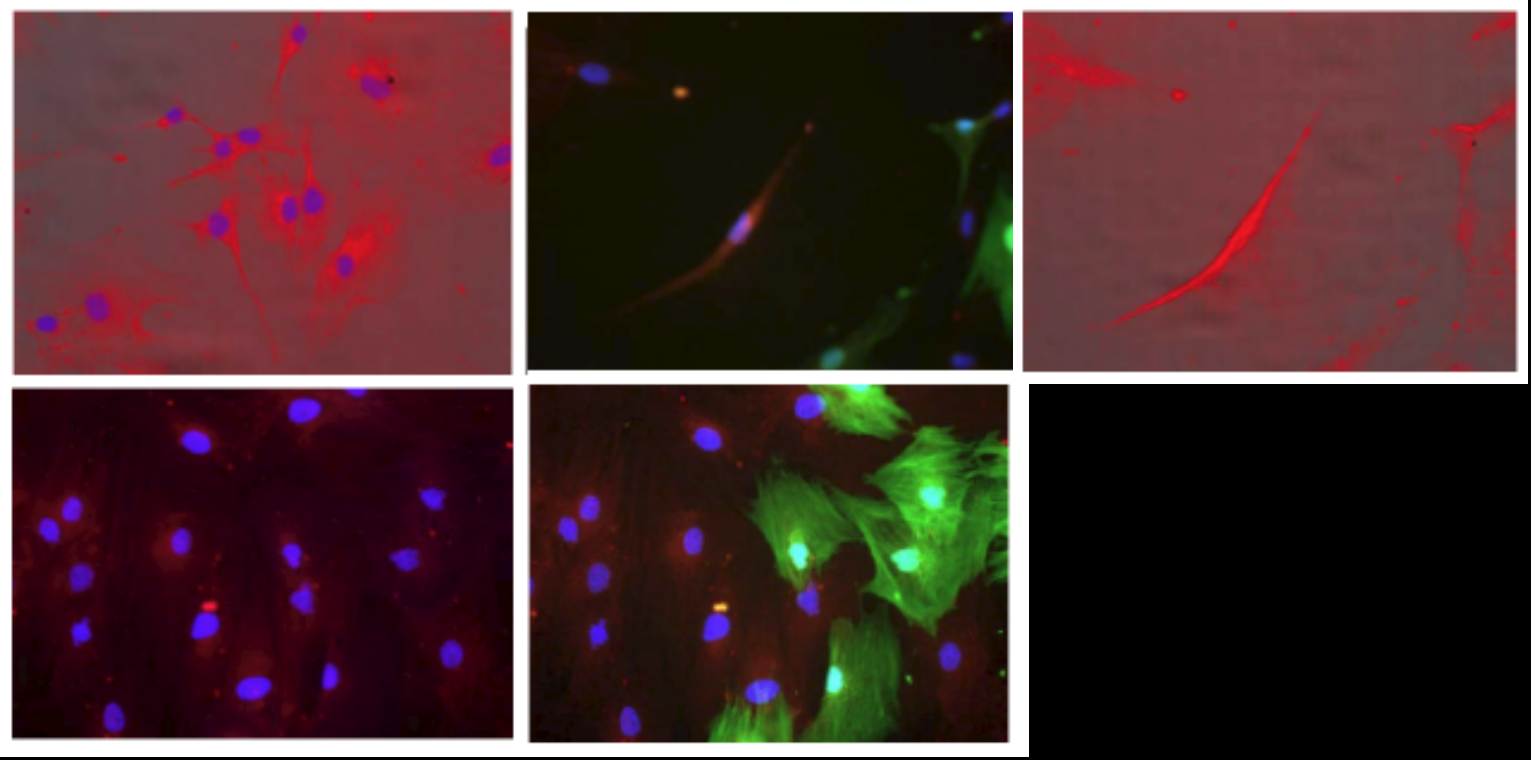
Control – untreated. Ad-HMSCs transduced with pCMV-Gluc-GFP lenti without further treatment



Osteogenic
Towards osteocytes?

targetingsystems

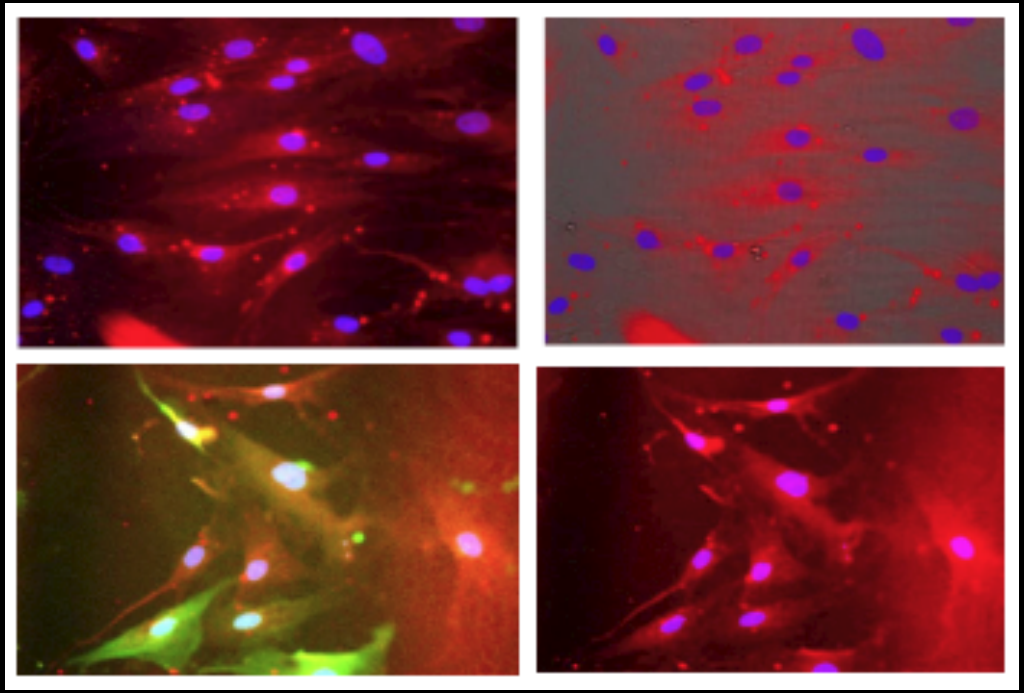
Differentiation of Ad-HMScs into islet cells

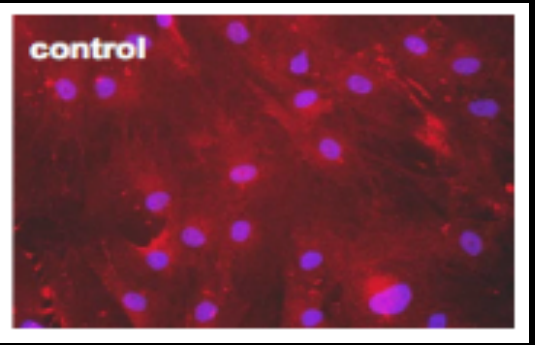


Differentiation of adipose tissue -derived mesenchymal stem cells into pancreatic Beta cells (top panel) Bottom panel (negative control). Staining for insulin C peptide, an islet sp marker

targetingsystems

Differentiation of Ad-HMSCs into skeletal muscle cells

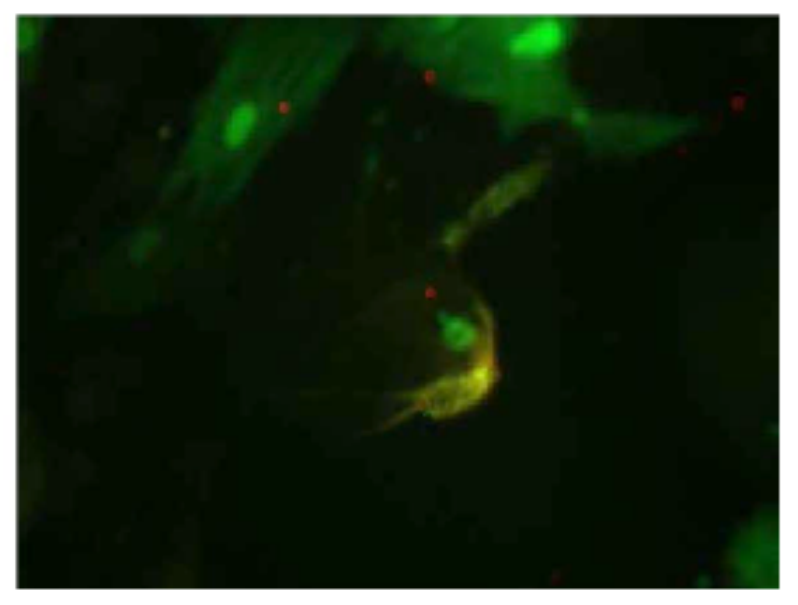




Cells were stained with antibodies to skeletal muscle troponin T, a marker for skeletal muscle cells

Differentiation of AD-HMScs into osteocytic lineage

Cells stained for BMP-2 (Bone morphogenetic protein) after treatment (controls were clearly negative hence not shown)



IP Summary

Patent #	Title
WO2011025980A1	Modified luciferase and uses thereof (granted)
WO2006/096735 A2	Enhancing A Luminescent Signal (granted)
US20080274485	Enhancement of a luminescent signal (granted)
US2007/0190587A1	Isolated Luciferase Gene of L. Italica (granted and licensed from Connecticul college exclusive worldwide)

IP Summary

	Title
WO28144052A3	Bioluminescent Imaging of Stem Cells
WO/2009/073523	De-Differentiation of Human Cells
US 5635380	Enhancement of nucleic acid transfer by coupling virus to nucleic acid via lipids (granted)

Options for bioluminescent cell lines

PROMOTER OPTIONS FOR EXPRESSION OF LUCIFERASES:

CMV Promoter
UBC promoter

NF-KB responsive promoter (NF-Kappa B)
This cell line can be used for the *in vitro* evaluation of natural compounds and *in vivo* optical imaging of tumor necrosis factor TNFα-induced NF-κB activation.

HIF- responsive promoter (Hypoxia inducible factor)

HIF activity is involved in angiogenesis required for cancer tumor growth, so HIF inhibitors such are (since 2006) under investigation for anti-cancer effects.[1]

P53 responsive promoter