KATHOLIEKE UNIVERSITEIT LEUVEN

Reporter gene imaging

WMIC 2012 Dublin educational program

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Contents

3 year educational program on 'biologicals':

1. Reporter gene systems: enzymes, transporters and receptors.

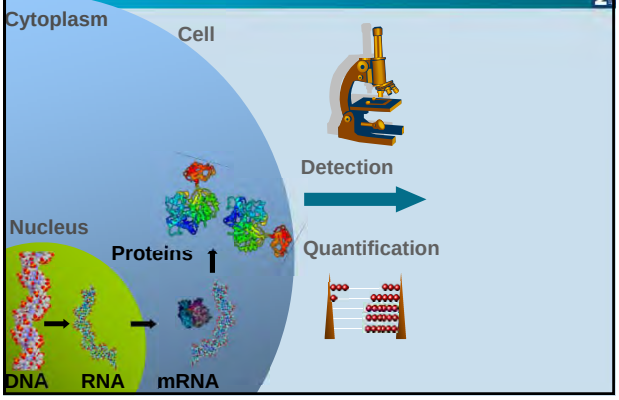
2. How to introduce reporter genes?

3. Reporter gene animals.

Reporter gene systems

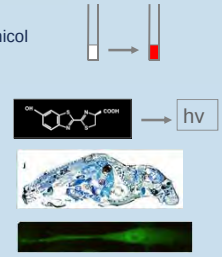
- Overall main focus: Learn about the diversity of biologicals in nature and their potential role as a tool for in vivo molecular imaging.
- Reporter gene systems: enzymes, transporters and receptors.
- Overview of the reporter gene systems
- Preclinical applications of cell tracking using reporter gene imaging
- Clinical translation of reporter gene imaging

Reporter Gene



Reporter Genes

- Gene encoding an easily detectable protein
- Colorimetric reaction: CAT (chloramfenicol acetyl transferase), β-lactamase
- Luminescent reaction: Luciferase
- Colorimetric reaction *in situ*: LacZ
- Fluorescent Proteins



The Ideal Reporter Gene

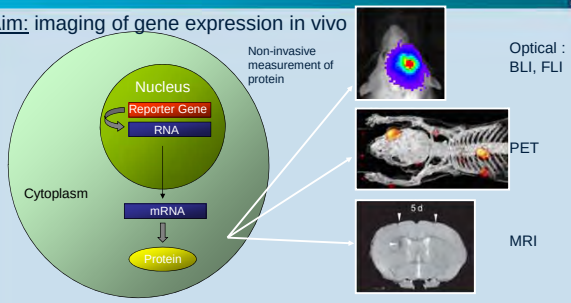
- Non-immunogenic

=> Present in mammalian cells, but not expressed.

- Non-toxic
- No significant biological effect on the cells in which expressed
- Size small enough to fit into a delivery vehicle
- Biological half-life of the reporter protein is relevant for the studied phenomenon.
- Reporter probe:
  - Reporter probe accumulates only where reporter gene is expressed.
  - No accumulation when the reporter gene is not expressed.
  - Stable in vivo and not be metabolized before reaching its target.
  - Rapidly clears from the circulation and doesn't interfere with detection of specific signal.
  - Probe or its metabolites should not be cytotoxic
  - Penetrates through biological barriers so that it can reach its target.
- The image signal should correlate well with levels of reporter gene mRNA and protein in vivo.
- Quantitative, high resolution, cheap, widespread imaging modality.

Imaging Reporter genes

Aim: imaging of gene expression in vivo



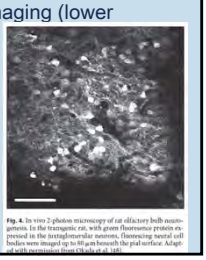
➔ Novel applications: imaging of gene regulation, imaging of protein-protein interactions, stem cell tracking, …

Advantages of non-invasive imaging

- Serial follow-up
- Less biological variation
- Fewer animals needed
- Statistical advantage
- Selection of animals for further interventions
- Intervention control for long term experiments

Fluorescent imaging

- excitation of a fluorophore by externally applied light (excitation photons) which results in the emission of light at a longer (less energetic) wavelength (emission photons)
- Intravital microscopy (invasive, small area of view)
- Macroscopic tomographic fluorescent Imaging (lower spatial resolution ~1mm)
- Limitations :
  - Limited depth of imaging
  - Autofluorescence
  - Photobleaching



Walczak 2007, Neurodegener. Diseases, 4:306-313

Fluorescent imaging reporters

Table 1 | Properties of the best FP variants[a,b]

Class	Protein	Source laboratory (references)	Excitation[c] (nm)	Emission[d] (nm)	Brightness[e]	Photostability[f]	pKa	Oligomerization
Far-red	mPlum[g]	Tsien (5)	590	649	4.1	53	<4.5	Monomer
Red	mCherry[g]	Tsien (4)	587	610	16	96	<4.5	Monomer
	tdTomato[g]	Tsien (4)	554	581	95	98	4.7	Tandem dimer
	mStrawberry[g]	Tsien (4)	574	596	26	15	<4.5	Monomer
	J-Red[h]	Evrogen	584	610	8.8*	13	5.0	Dimer
	DsRed-monomer[h]	Clontech	556	586	3.5	16	4.5	Monomer
Orange	mOrange[g]	Tsien (4)	548	562	49	9.0	6.5	Monomer
	mKO	MBL Intl. (10)	548	559	31*	122	5.0	Monomer
Yellow-green	mCitrine[i]	Tsien (16,23)	516	529	59	49	5.7	Monomer
	Venus	Miyawaki (1)	515	528	53*	15	6.0	Weak dimer[j]
	YPet[g]	Daugherty (2)	517	530	80*	49	5.6	Weak dimer[j]
	EYFP	Invitrogen (18)	514	527	51	60	6.9	Weak dimer[j]
Green	Emerald[g]	Invitrogen (18)	487	509	39	0.69[k]	6.0	Weak dimer[j]
	EGFP	Clontech[l]	488	507	34	174	6.0	Weak dimer[j]
Cyan	CyPet	Daugherty (2)	435	477	18*	59	5.0	Weak dimer[j]
	mCFPm[m]	Tsien (23)	433	475	13	64	4.7	Monomer
	Cerulean[g]	Piston (3)	433	475	27*	36	4.7	Weak dimer[j]
UV-excitable green	T-Sapphire[g]	Griesbeck (6)	399	511	26*	25	4.9	Weak dimer[j]

Shaner et al., Nat Methods 2005

Bioluminescence

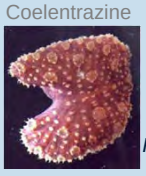
- Light producion by a chemical reaction which occurs in a living organism.
- Occurs in many organisms including: Bacteria,Fungi,Dinoflagellates, Mollusca, Annelid worms (Earthworms), Crustaceans, Fish, Insects
- Primarily marine phenomenon
- Rather rare phenomenon on land
  - Fungi
  - Fireflies (e.g. Photinus Pyralis)
- (There are no luminous "flowering" plants, birds, reptiles, amphibians or mammals.)

Bioluminescence

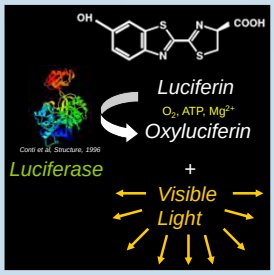
North American Firefly

D-Luciferin

Coelentrazine

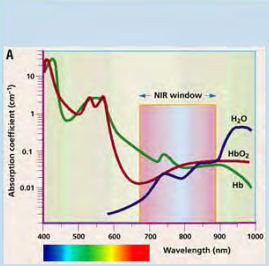


Gaussia Princeps

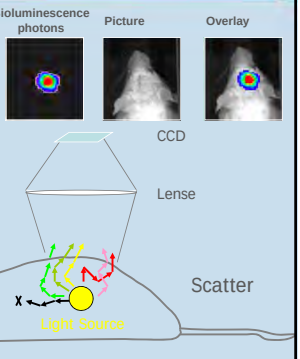


Renilla Reniformis

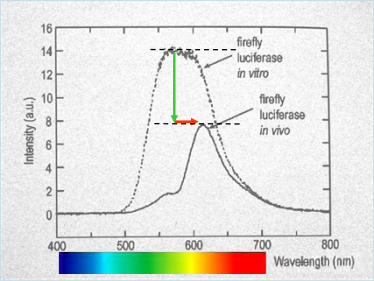
Interactions of visible light photons in biological tissue



Absorption



Spectrum of *in vivo* Measured Light



Attenuated

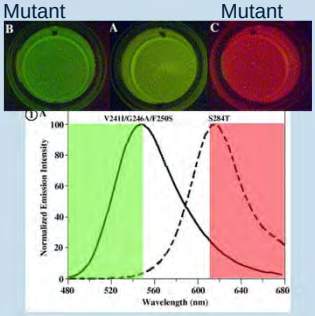
Red-shifted

Rice et al, J Biomed Opt, 2001

The Hunt for the Red Luciferase

Green Mutant | WT FLuc | Red Mutant

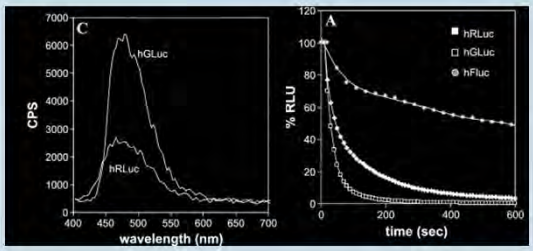
Val241Ile
Gly246Ala
Phe250Ser



Ser284Thr

Branchini et al, Anal Biochem, 2005

Gaussia & *Renilla*, the blue luciferases



Tannous et al., Hum Gene Ther 2005

Engineering Challenge	Design Feature
Living tissues strongly absorb blue and green light	CCD is highly sensitive in red range
Camera must act as a sensitive photon detector as well as a "standard camera"	Wide dynamic range
Low light levels and high sensitivity require minimized background light	Optimized imaging chamber
Low light levels and high sensitivity require a camera with low noise	Cooled CCD, low-noise electronic readout

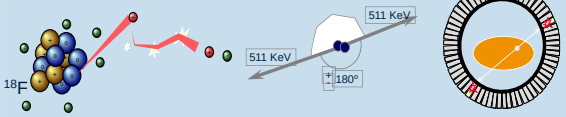
Xenogen info@xenogen.com

Positron Emission Tomography

PET imaging

- Principle: detection of highly energetic photons by PET camera.
- Photons result from probes (or tracers) that have been injected into the subject.
- The probes contain a radioactive isotope that decays and emits the photons detected by the camera.
- The image obtained depicts the biodistribution of the probe. Different probes show different molecular events.
- Biological properties of the probe determine the meaning of the image.

Positron Emission Tomography

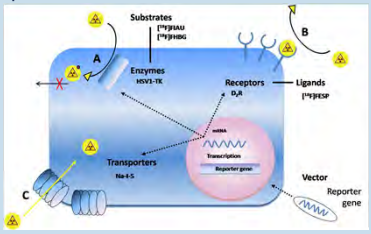


PET probes

- Use of ^{15}O, ^{13}N, ^{11}C, ^{18}F (substitute for H)= “molecules of life”
- Others: ^{64}Cu, ^{62}Cu, ^{124}I, ^{68}Ga
- Most produced by in site cyclotron
- Relatively short half lives ⇒ daily scans feasible
- Wide variety of tracers described in literatures, but only limited number of tracers available per site.

PET Reporter genes

- PET Reporter gene: gene coding for a protein that can be dected and quantified by a PET probe.
- Three categories of proteins
  - Enzymes
  - Receptors
  - Transporters

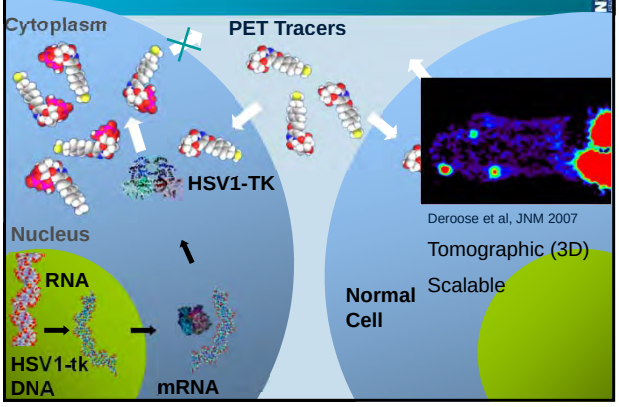


- Limitations : penetrance of tracers through blood-brain barrier

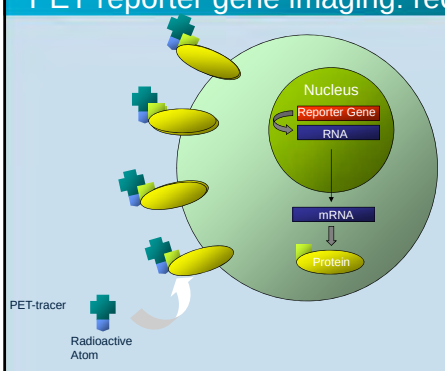
PET reporter genes

- Herpes Simplex Virus 1 Thymidine Kinase (HSV1-TK) (+ mutant sr39TK)
- Hypoxanthine Phosphoribosyl Transferase
- L Amino Acid Decarboxylase
- Dopamine 2 receptor (D2R + mutant D2RA80)
- Somatostatin Receptor
- Estrogen receptor (hERL)
- Dopamine Transporter
- Sodium Iodide Symporter
- Catecholamine Transporter

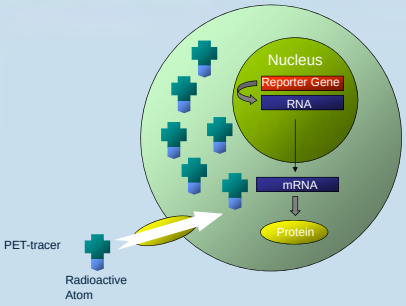
HSV1-tk Reporter Gene Imaging



PET reporter gene imaging: receptor



PET reporter gene imaging: transporter



Magnetic resonance imaging (MRI)

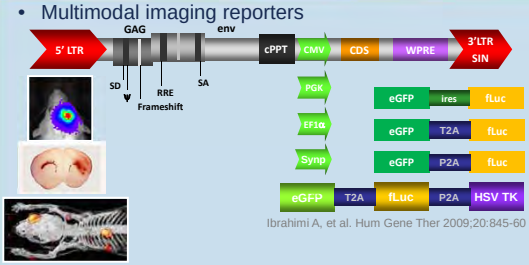
- radiofrequency electromagnetic waves that are emitted by atoms when they return to a basic aligned state within a magnetic field
- High spatial resolution (10-100 µm)
- Low sensitivity
- Quantification difficult (negative contrast agents)

MRI reporter genes

- Tyrosinase :
  - enzyme required for melanin synthesis; melanin :
  - ⊖ toxicity (reactive oxygen species)
- Transferrin receptor :
  - binds endogenous transferrin + 2 iron atoms
- Ferritin :
  - Storage of endogenous iron (4000 iron atoms in crystal configuration
  - ⊕ absence of reporter probe
  - ⊖ sensitivity (magnet strength, random orientation of spins in crystal), different iron concentrations/tissue, does not report cell viability, quantification difficult
- MagA :
  - Encodes a H+/Fe(II) antiporter
  - Synthesis of bacterial magnetosomes = magnetic iron-oxide nanoparticles = tiny magnets produced by naturally occurring magnetotactic bacteria

Technical developments

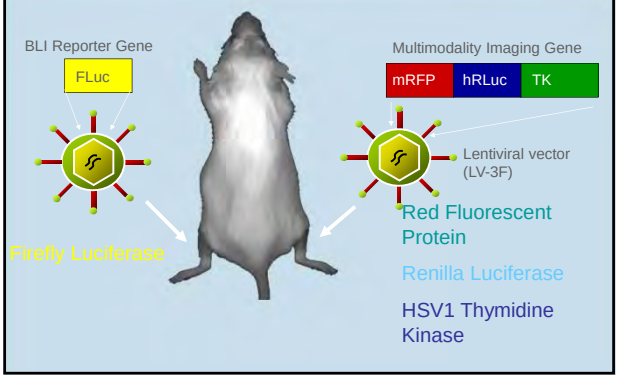
- Multimodal imaging reporters



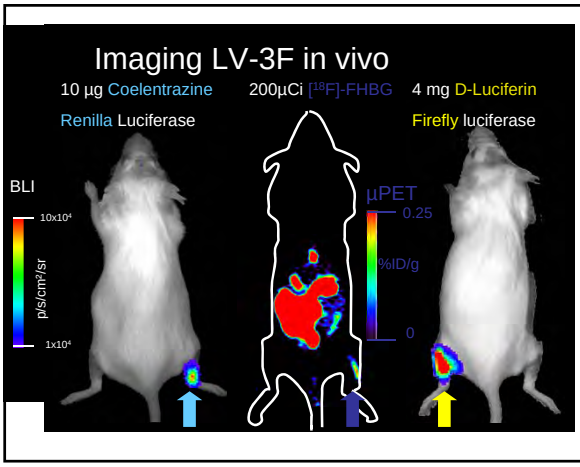
Ibrahimi A, et al. Hum Gene Ther 2009;20:845-60

- New reporter development and evaluation
  - Evaluation of ferritin as MRI reporter (Vande Velde et al.2011, Gene Ther.; Vande Velde et al., 2012, Neuroimage)
  - Evaluation of Cannabinoid Receptor Type 2 as a new PET Reporter Gene (Vandeputte et al., 2011, J. Nucl Med., 52(7):1102-9)

Multimodality imaging reporter gene (3F)



Imaging LV-3F in vivo

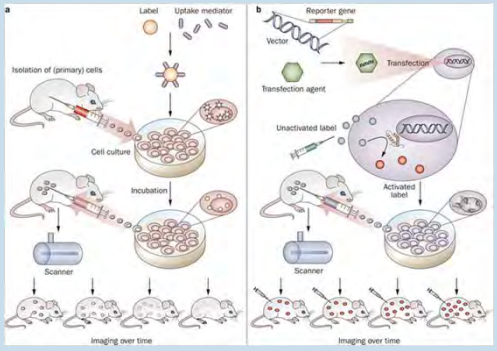


Preclinical application for reporter genes

- Cell labelling/tracking/fate determination
- Promoter activity
- Cis-regulatory elements
- Transcription factors
- In vitro gene transfer assay
- Gene therapy monitoring
- Protein trafficking (fusion genes)
- Protein/protein interactions

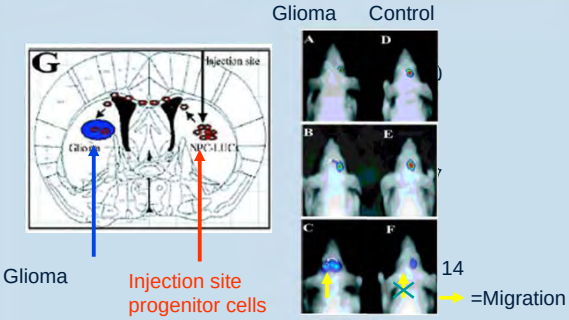
Monitoring cell based therapies

Principles of direct and indirect labeling methods for cell tracking



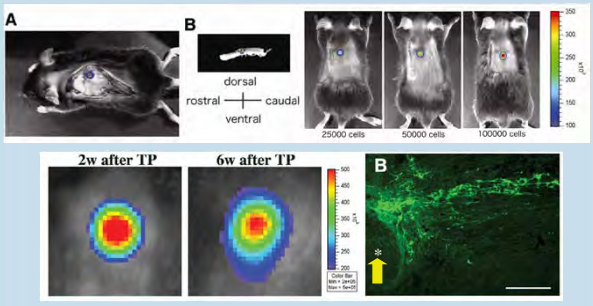
Kircher, M. F. *et al.* (2011) *Nat. Rev. Clin. Oncol.*

Migration of neural progenitor cells to glioblastoma



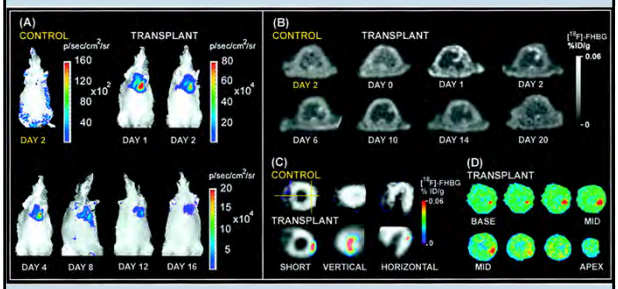
Tang et al., Hum. Gene Ther. 2003

Neural Progenitor Cell Therapy for Spinal Cord Injury



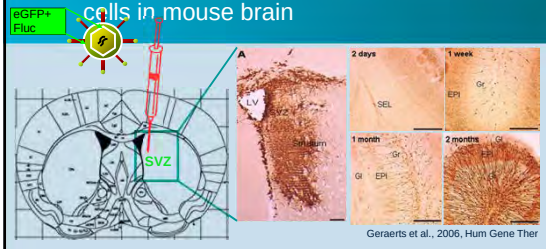
Okada et al., Faseb J 2005

Bimodality PET and BLI imaging of cardiac cellular therapy



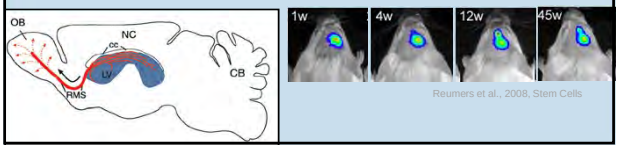
Wu et al., Circulation 2003

Labeling and BLI of endogenous neural stem cells in mouse brain



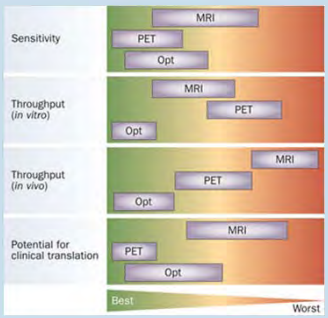
Geraerts et al., 2006, Hum Gene Ther

Migration/differentiation/ integration of labelled cells



Reumers et al., 2008, Stem Cells

Characteristics of modalities for cell tracking

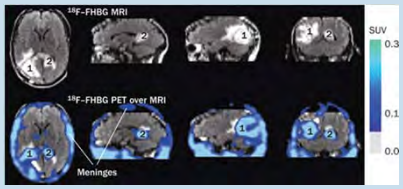


Kircher, M. F. *et al.* (2011) *Nat. Rev. Clin. Oncol.*

Clinical translation of reporter gene imaging

- 2009 : first use of reporter-gene technology to image therapeutic cells in a human

Figure Brain MRI with and without superimposed PET of a patient after intracranial infusions of autologous cytolytic T cells expressing IL-13 zetakine and herpes simplex virus 1 thymidine kinase



Yaghoubi, S. S. *et al. Nat. Clin. Pract. Oncol.* **6**, 53–58 (2009)

Further reading - acknowledgements

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- Vande Velde et al., 2009, Magnetic resonance imaging and spectroscopy methods for molecular imaging. *Q J Nucl Med Mol Imaging*.;53(6):565-85
- Kircher M et al., 2011, Noninvasive cell-tracking methods. Nature Rev Clin Oncol. 8, 677-688

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