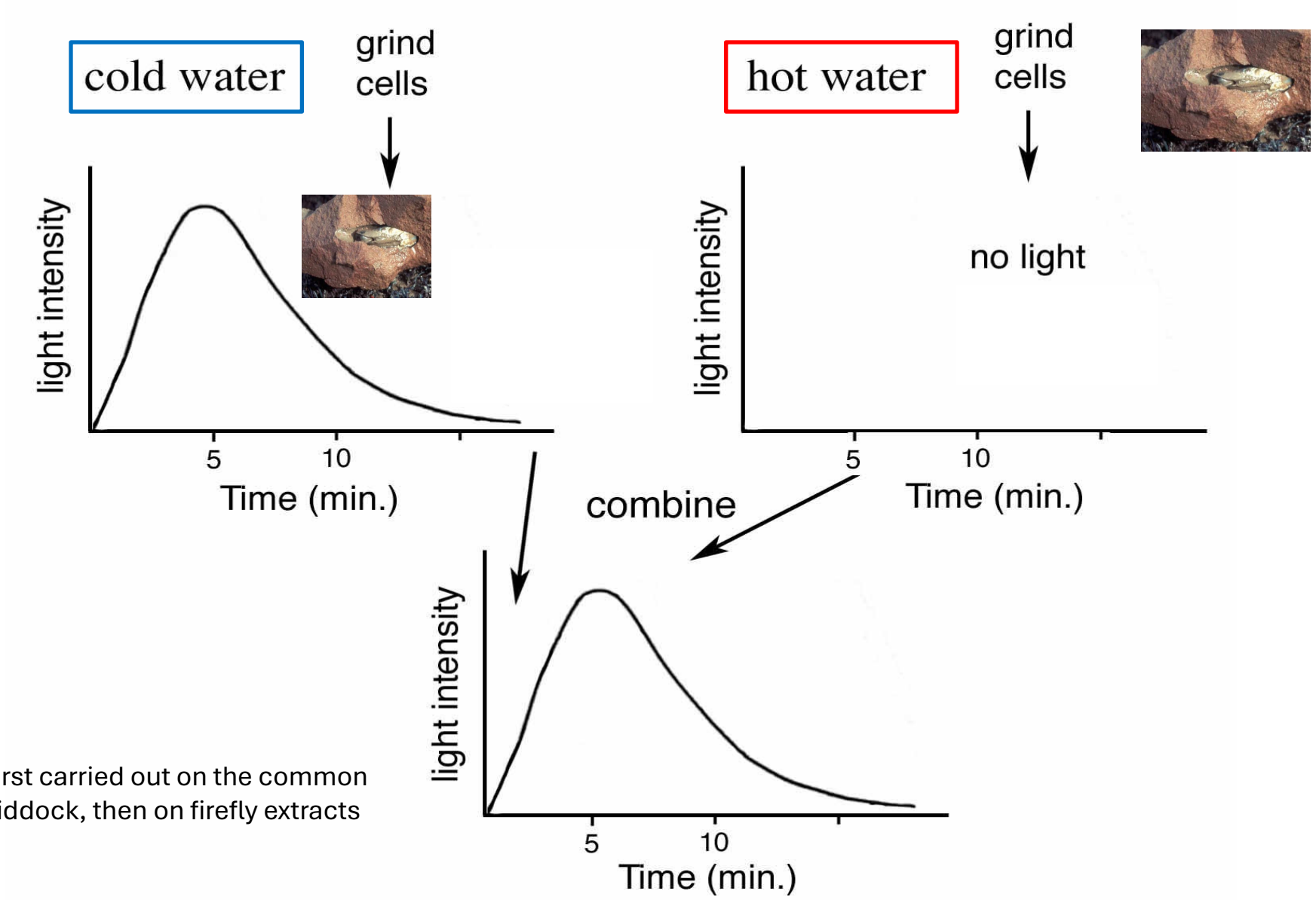
Luciferins and luciferases

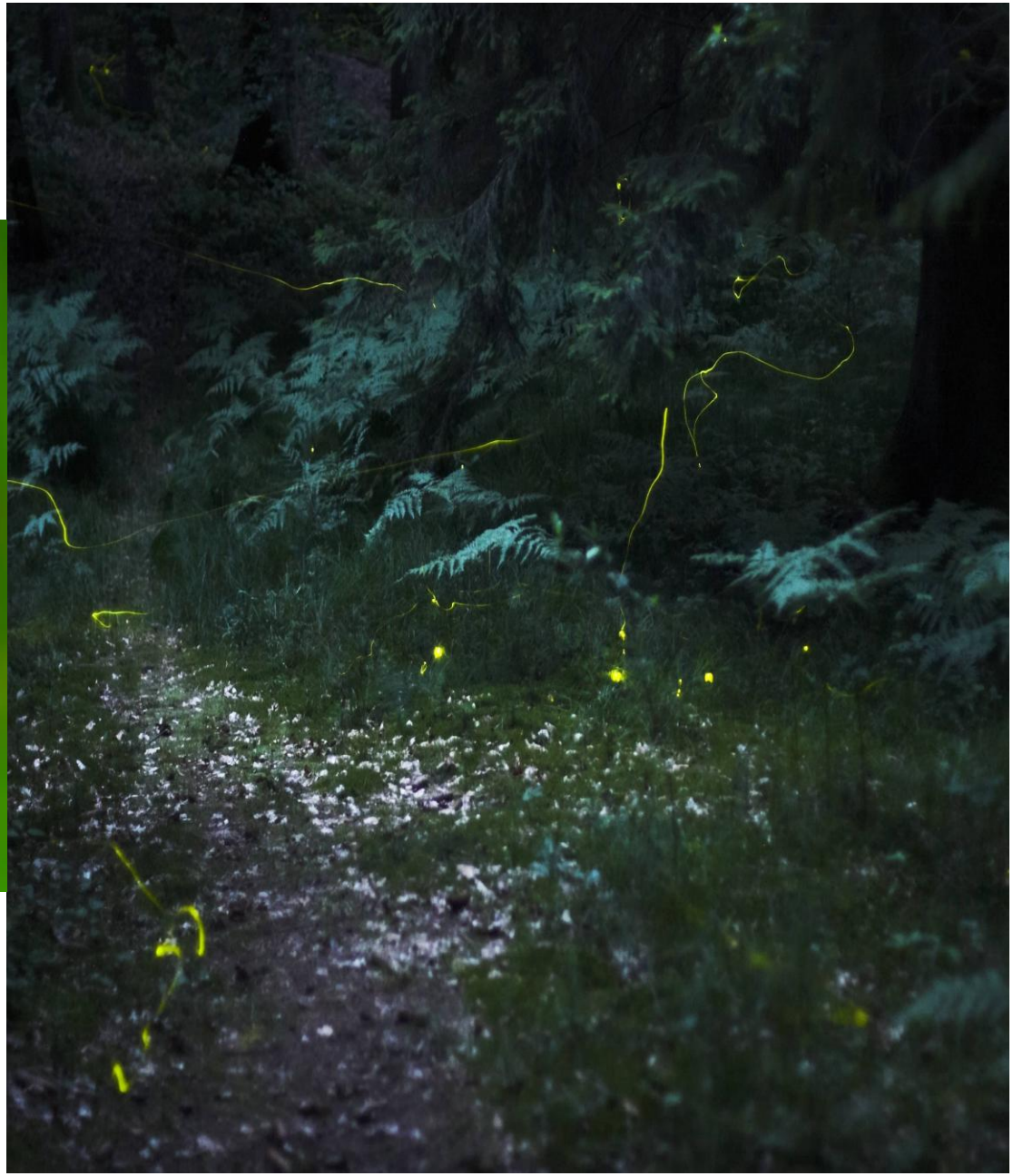
Pholas dactylus
The common piddock

A «bioluminescent» clam that drills its way into rock

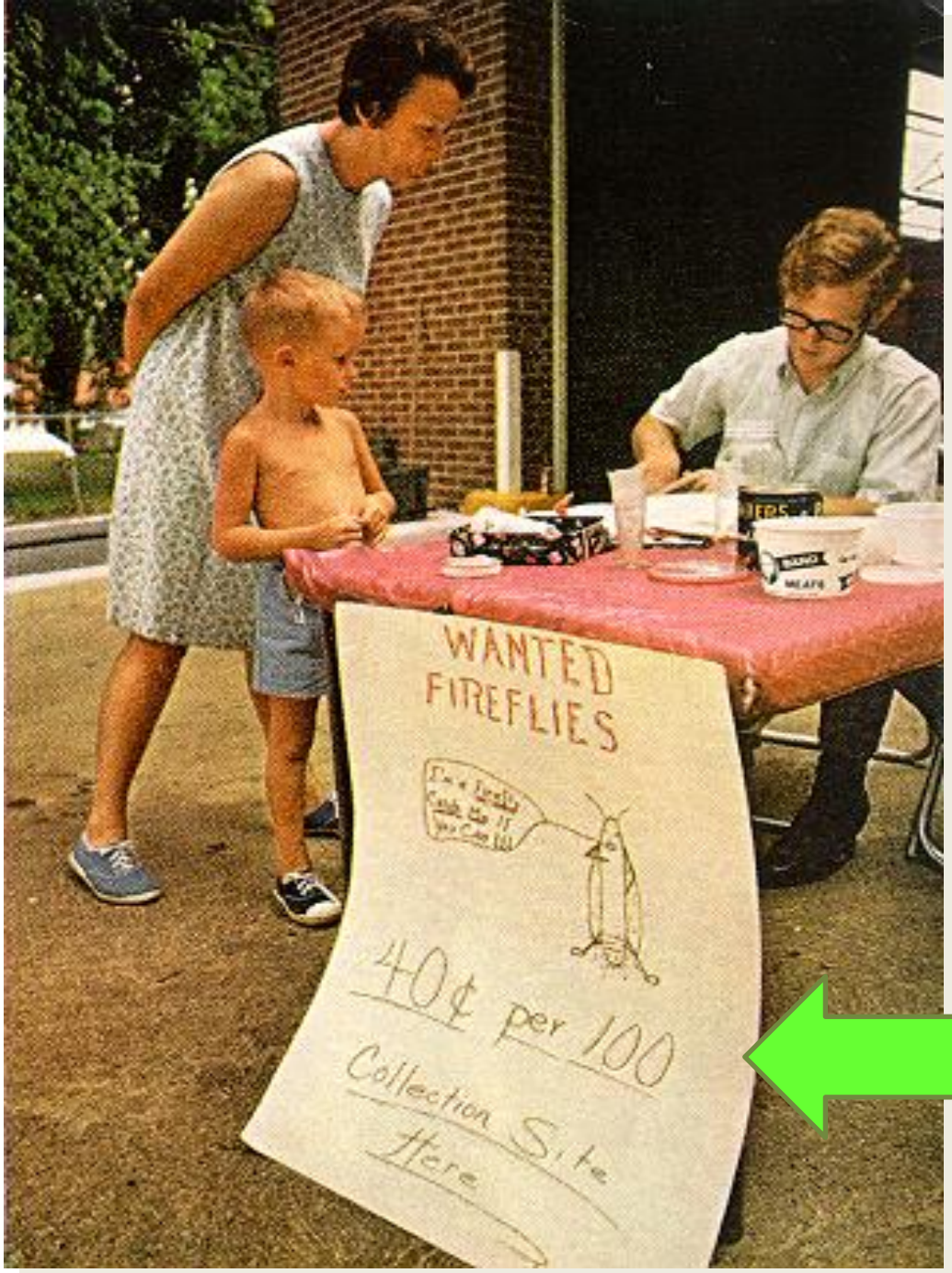
The luciferin (substrate) / luciferase (enzyme) reaction (Dubois 1885-7)

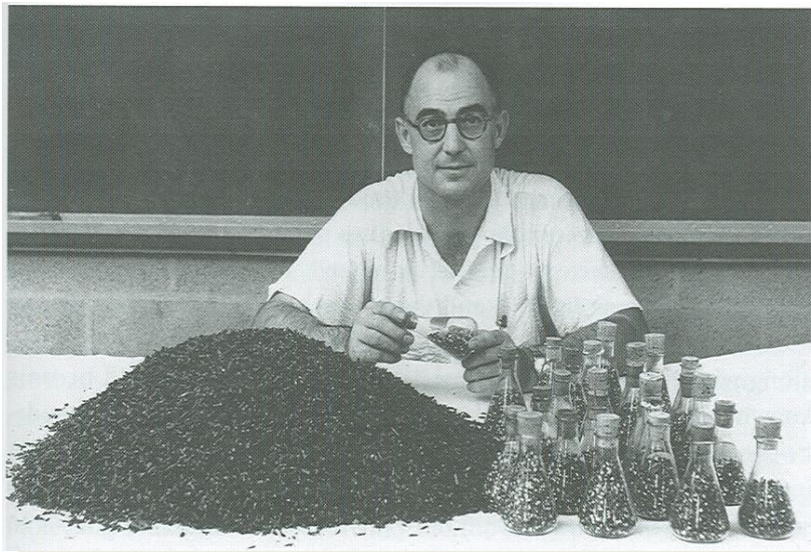


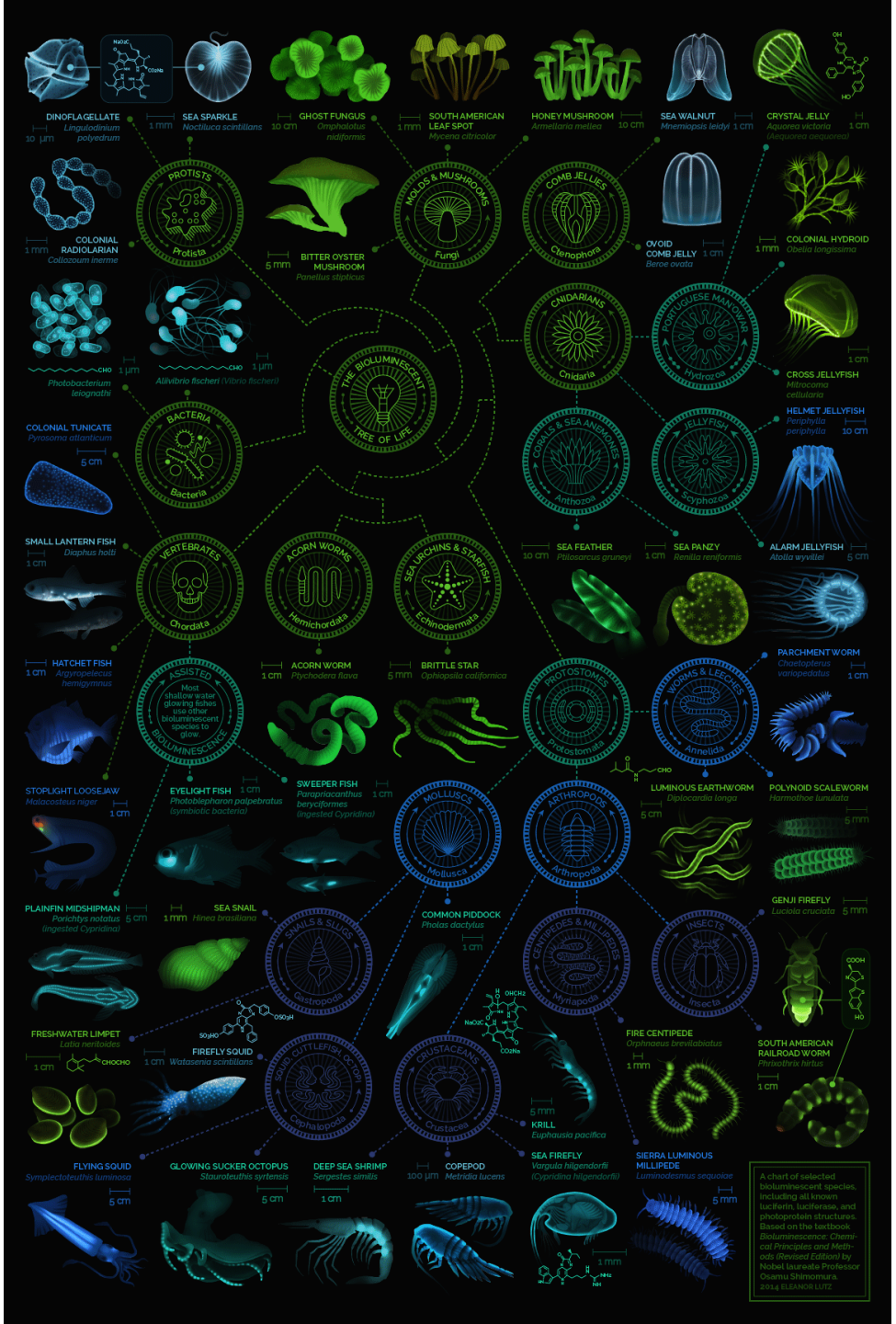
First carried out on the common piddock, then on firefly extracts



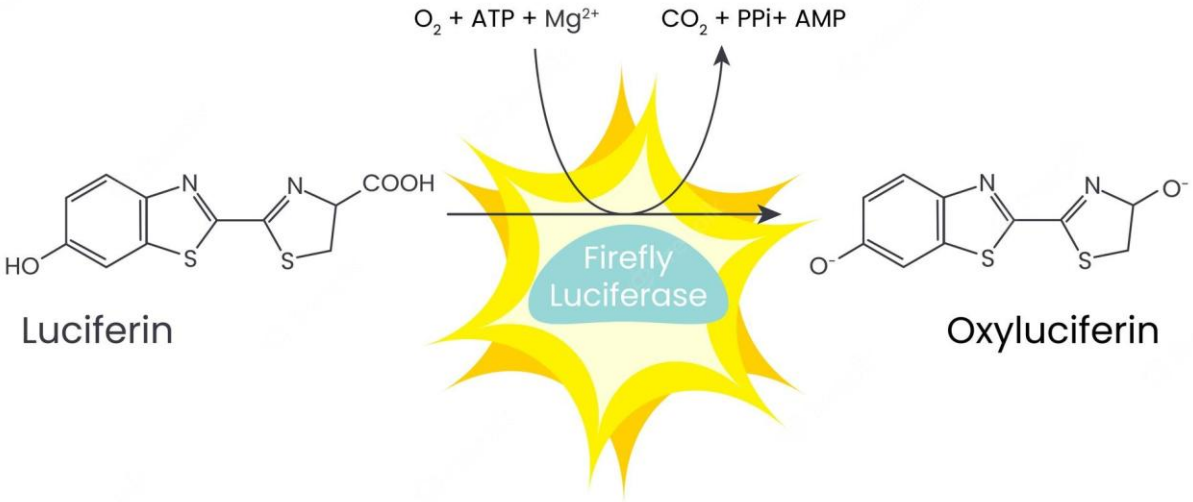
Understanding luciferase






THE BIOLUMINESCENT TREE OF LIFE
PROTISTS
Protista
MOLDS & MUSHROOMS
Fungi
COMB JELLIES
Ctenophora
CNIDARIANS
Cnidaria
PORTUGUESE MAN O' WAR
Hydrozoa
BACTERIA
Bacteria
CORALS & SEA ANEMONES
Anthozoa
JELLYFISH
Scyphozoa
VERTEBRATES
Chordata
ACORN WORMS
Hemichordata
SEA URCHINS & STARFISH
Echinodermata
PROTOSTOMES
Protostomia
WORMS & LEECHES
Annelida
MOLLUSCS
Mollusca
ARTHROPODS
Arthropoda
SNAILS & SLUGS
Gastropoda
CENTIPEDES & MILLIPEDES
Myriapoda
INSECTS
Insecta
SQUID, CUTTLEFISH, OCTOPUS
Cephalopoda
CRUSTACEANS
Crustacea
ASSISTED BIOLUMINESCENCE
Most shallow water glowing fishes use other bioluminescent species to glow.
DINOFLAGELLATE
Lingulodinium polyedrum
10 µm
SEA SPARKLE
Noctiluca scintillans
1 mm
GHOST FUNGUS
Omphalotus nidiformis
10 cm
SOUTH AMERICAN LEAF SPOT
Mycena citricolor
1 mm
HONEY MUSHROOM
Armillaria mellea
10 cm
SEA WALNUT
Mnemiopsis leidyi
1 cm
CRYSTAL JELLY
Aequorea victoria (Aequorea aequorea)
1 cm
COLONIAL RADIOLARIAN
Collozoum inerme
1 mm
BITTER OYSTER MUSHROOM
Panellus stipticus
5 mm
OVOID COMB JELLY
Beroe ovata
1 cm
COLONIAL HYDROID
Obelia longissima
1 mm
Photobacterium leiognathi
1 µm
Aliivibrio fischeri (Vibrio fischeri)
1 µm
CROSS JELLYFISH
Mitrocoma cellularia
1 cm
HELMET JELLYFISH
Periphylla periphylla
10 cm
COLONIAL TUNICATE
Pyrosoma atlanticum
5 cm
ALARM JELLYFISH
Atolla wyvillei
5 cm
SEA FEATHER
Ptilosarcus gruneyi
10 cm
SEA PANZY
Renilla reniformis
1 cm
SMALL LANTERN FISH
Diaphus holti
1 cm
HATCHET FISH
Argyropelecus hemigymnus
1 cm
ACORN WORM
Ptychodera flava
1 cm
BRITTLE STAR
Ophiopsila californica
5 mm
PARCHMENT WORM
Chaetopterus variopedatus
1 cm
STOPLIGHT LOOSEJAW
Malacosteus niger
1 cm
EYELIGHT FISH
Photoblepharon palpebratus (symbiotic bacteria)
1 cm
SWEEPER FISH
Parapriacanthus beryciformes (ingested Cypridina)
1 cm
LUMINOUS EARTHWORM
Diplocardia longa
5 cm
POLYNOID SCALEWORM
Harmothoe lunulata
5 mm
PLAINFIN MIDSHIPMAN
Porichthys notatus (ingested Cypridina)
5 cm
SEA SNAIL
Hinea brasiliana
1 mm
COMMON PIDDOCK
Pholas dactylus
1 cm
GENJI FIREFLY
Luciola cruciata
5 mm
FRESHWATER LIMPET
Latia neritoides
1 cm
FIREFLY SQUID
Watasenia scintillans
1 cm
FIRE CENTIPEDE
Orphnaeus brevilabiatus
1 mm
SOUTH AMERICAN RAILROAD WORM
Phrixothrix hirtus
1 cm
KRILL
Euphausia pacifica
5 mm
FLYING SQUID
Symplectoteuthis luminosa
5 cm
GLOWING SUCKER OCTOPUS
Stauroteuthis syrtensis
5 cm
DEEP SEA SHRIMP
Sergestes similis
1 cm
COPEPOD
Metridia lucens
100 µm
SEA FIREFLY
Vargula hilgendorfii (Cypridina hilgendorfii)
1 mm
SIERRA LUMINOUS MILLIPEDE
Luminodesmus sequoiae
5 mm
A chart of selected bioluminescent species, including all known luciferin, luciferase, and photoprotein structures. Based on the textbook Bioluminescence: Chemical Principles and Methods (Revised Edition) by Nobel laureate Professor Osamu Shimomura.
2014 ELEANOR LUTZ

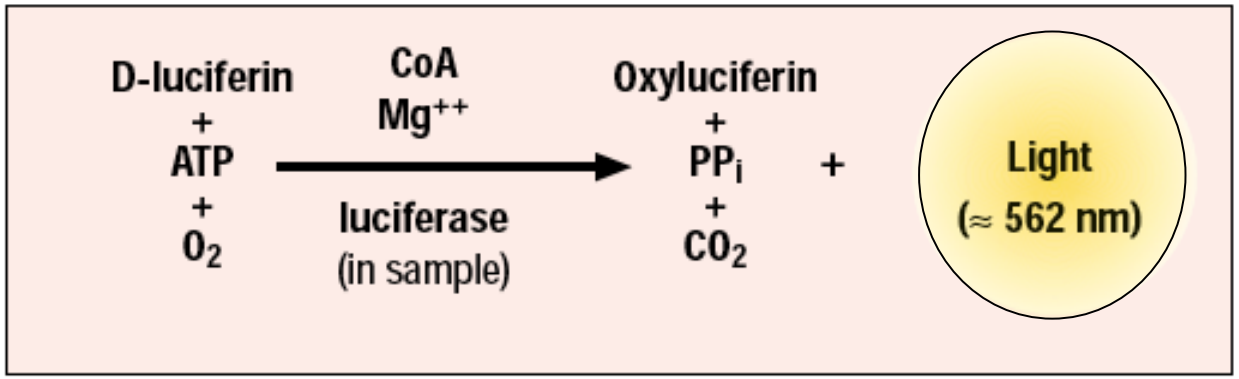
Bioluminescence

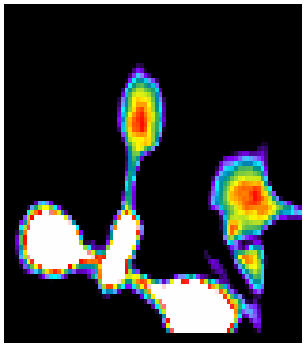


Luciferase:

Photinus pyralis

- **Firefly luciferase** (*Photinus pyralis)*
- Firefly luciferase produces light by **ATP-dependent oxidation**



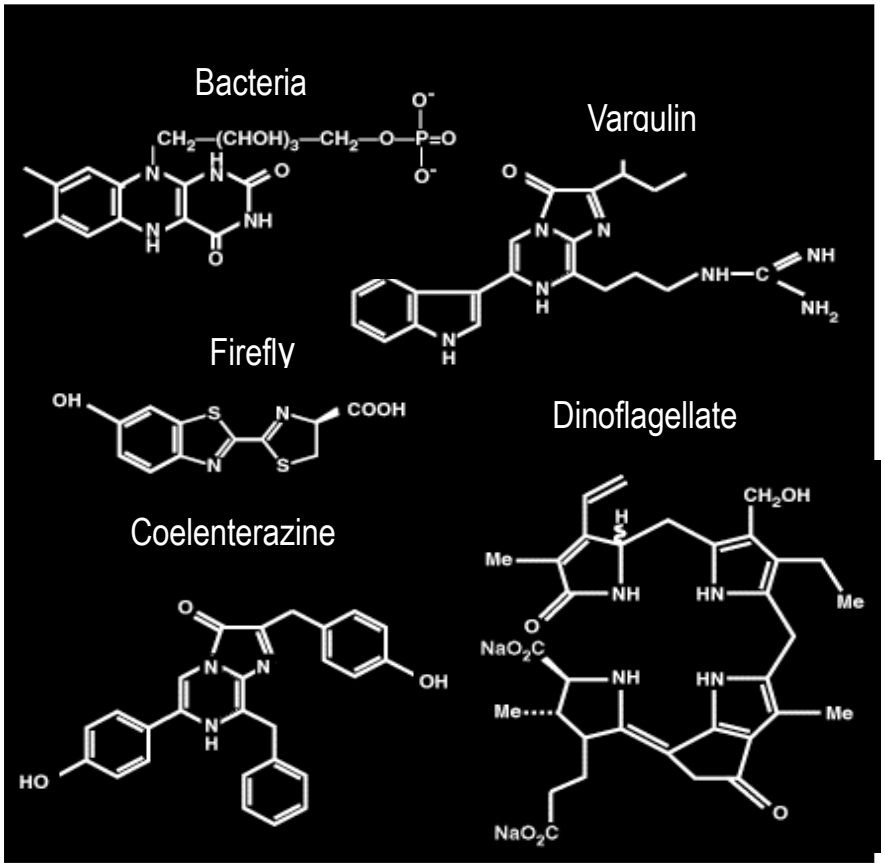


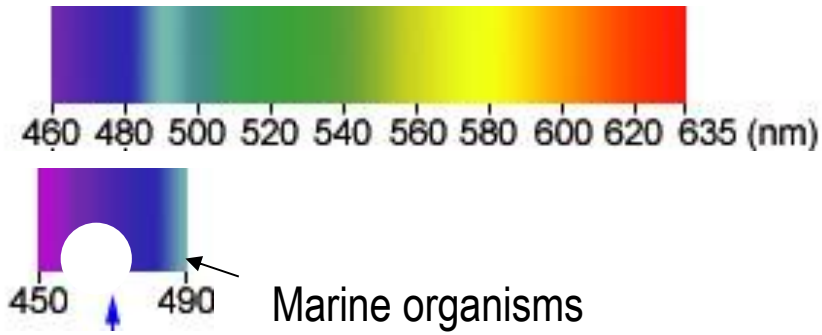
- **Bioluminescence** or light emission

is determined by a **luminometer**

Luciferin chemistry

- There are many different **luciferins,**
- each specific luciferin has its own special **luciferase.**

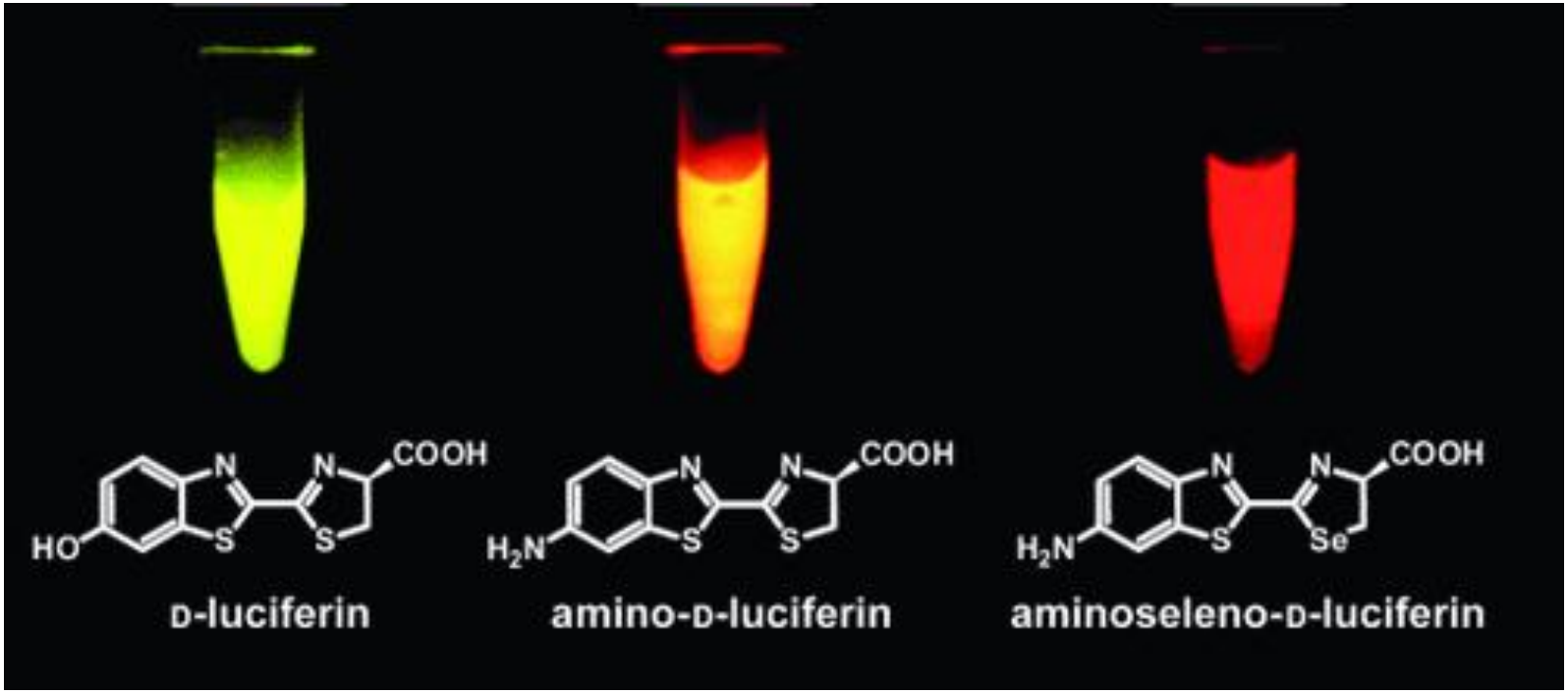




Emission maxima of most luminous **marine organisms 450-490 nm**

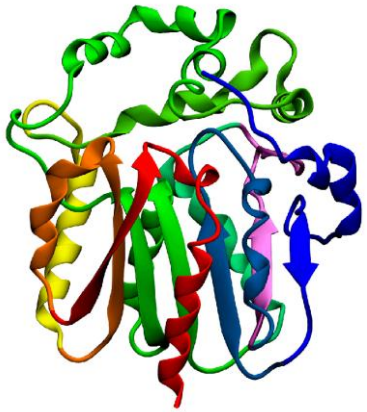
- Best Wavelenghth for light trasmission in ocean water
- Maximal sensitivity of visual pigments of most marine organisms

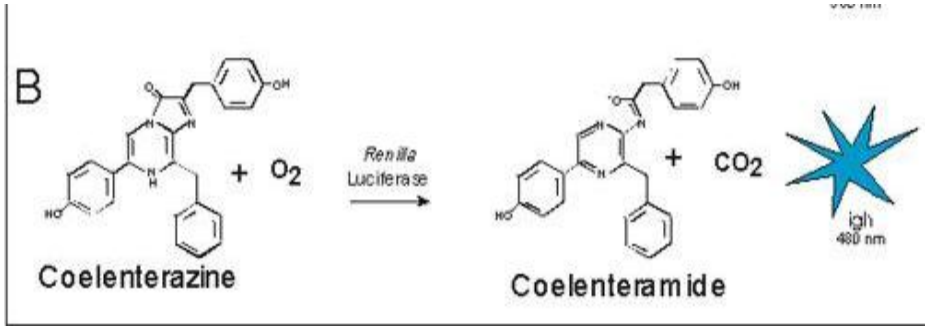
Luciferin chemistry

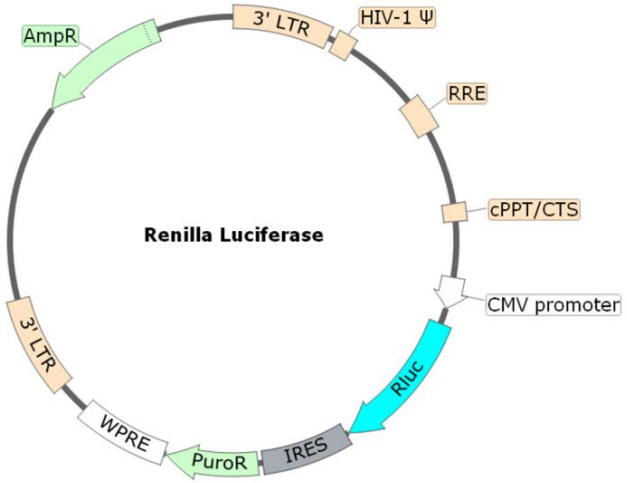


Renilla reniformis

è un celenterato coloniale

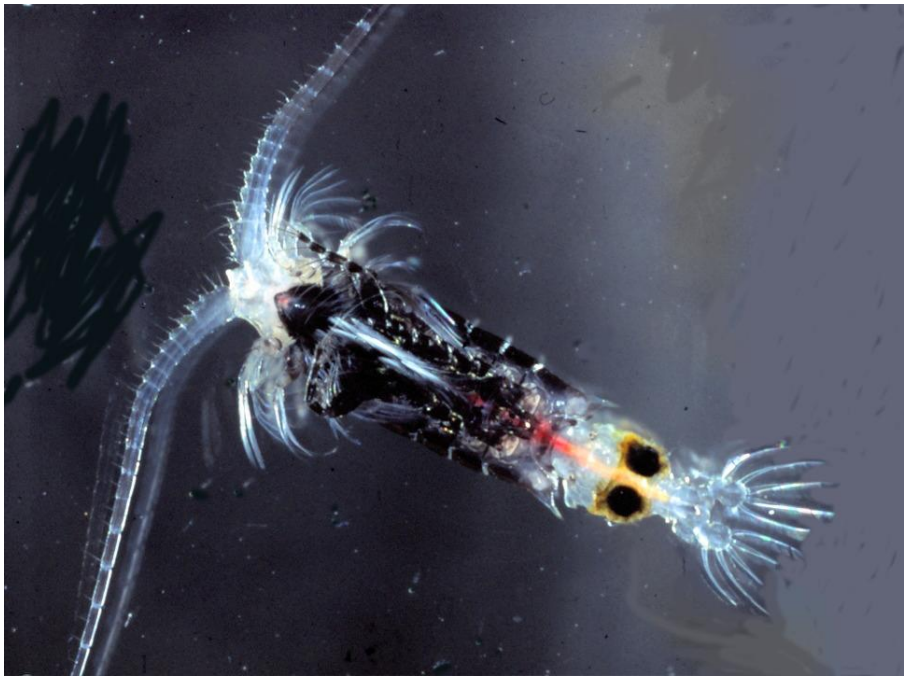


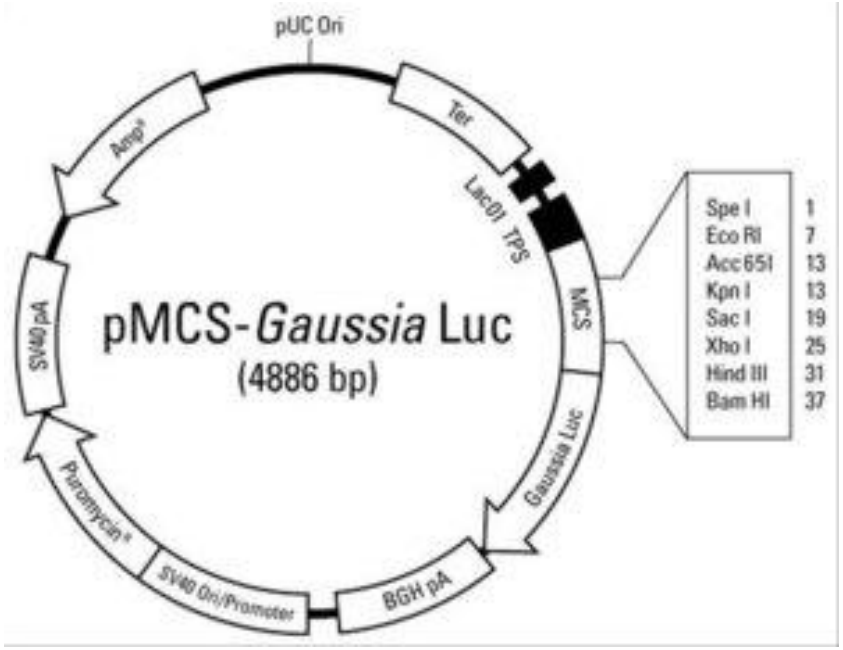


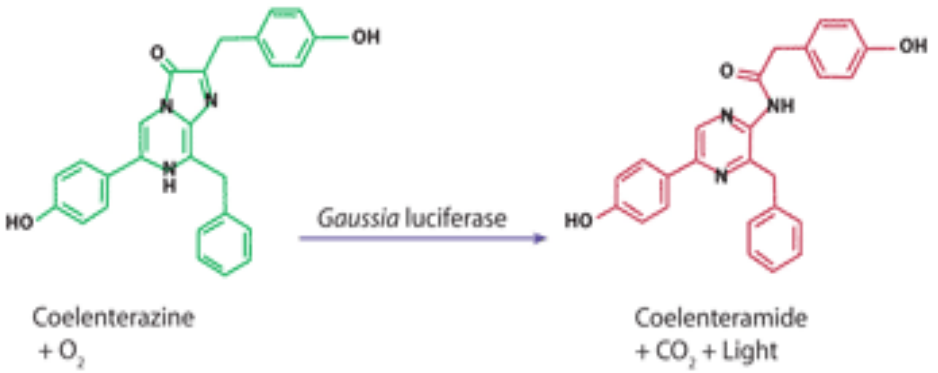


Gaussia Princeps

è un copepode mesopelagico
si trova nelle acque temperate e tropicali di tutto il mondo

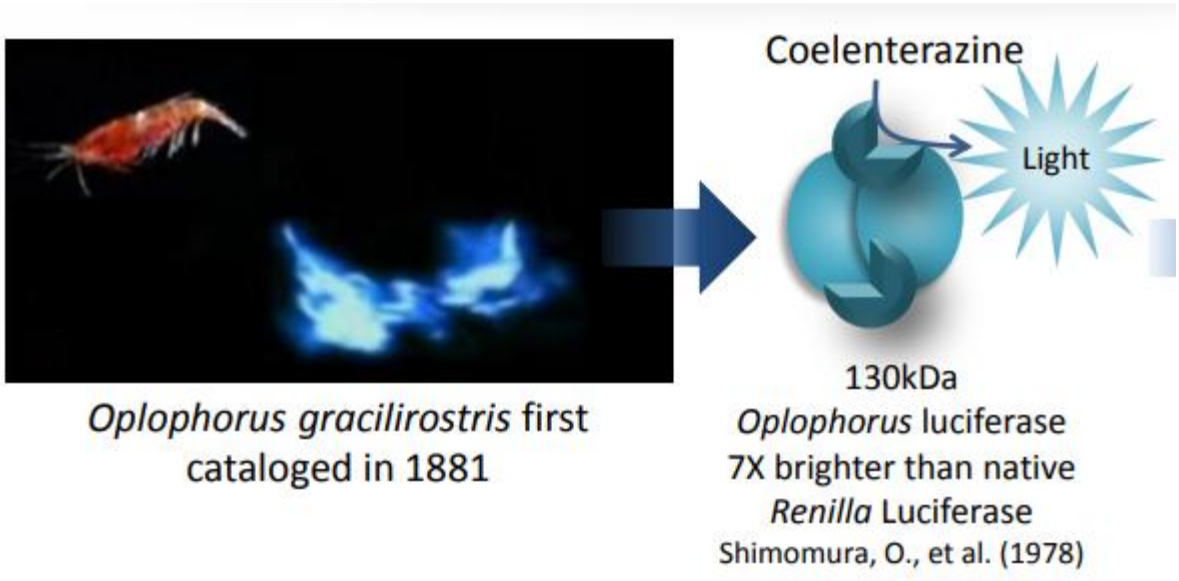






Problema biotecnologico

Considerate la luciferasi derivata dal gamberetto *Oplophorus gracilirostris*

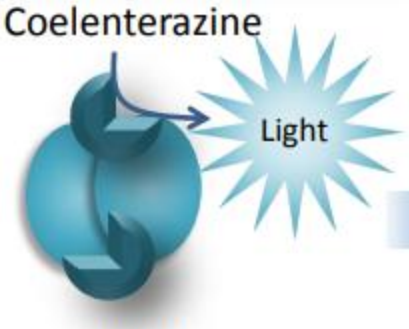


Proponete un approccio sperimentale per la sua «ottimizzazione» per l'uso in biologia cellulare

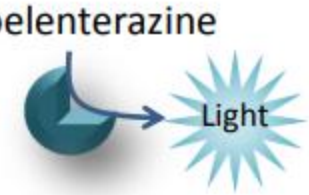
Evolution of NanoLuc from ocean to lab bench

Promega

Oplophorus gracilirostris first cataloged in 1881



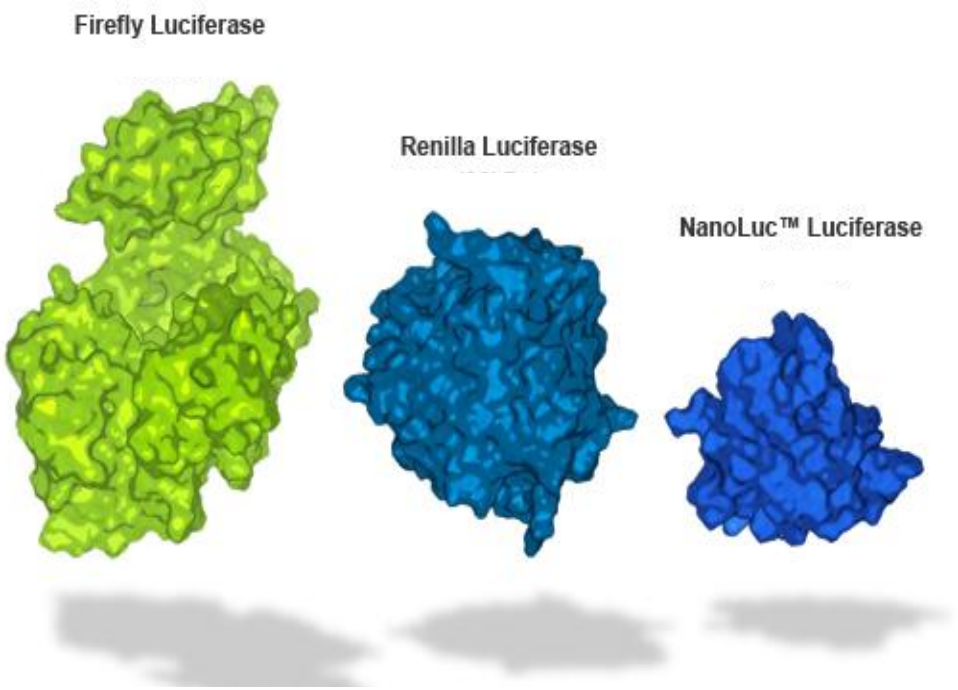
130kDa
Oplophorus luciferase
7X brighter than native *Renilla* Luciferase
Shimomura, O., et al. (1978)



19kOluc
19kDa subunit is catalytic.
Light output & stability compromised.
Inouye, S., et al. (2000)

Promega Advanced Technologies Group

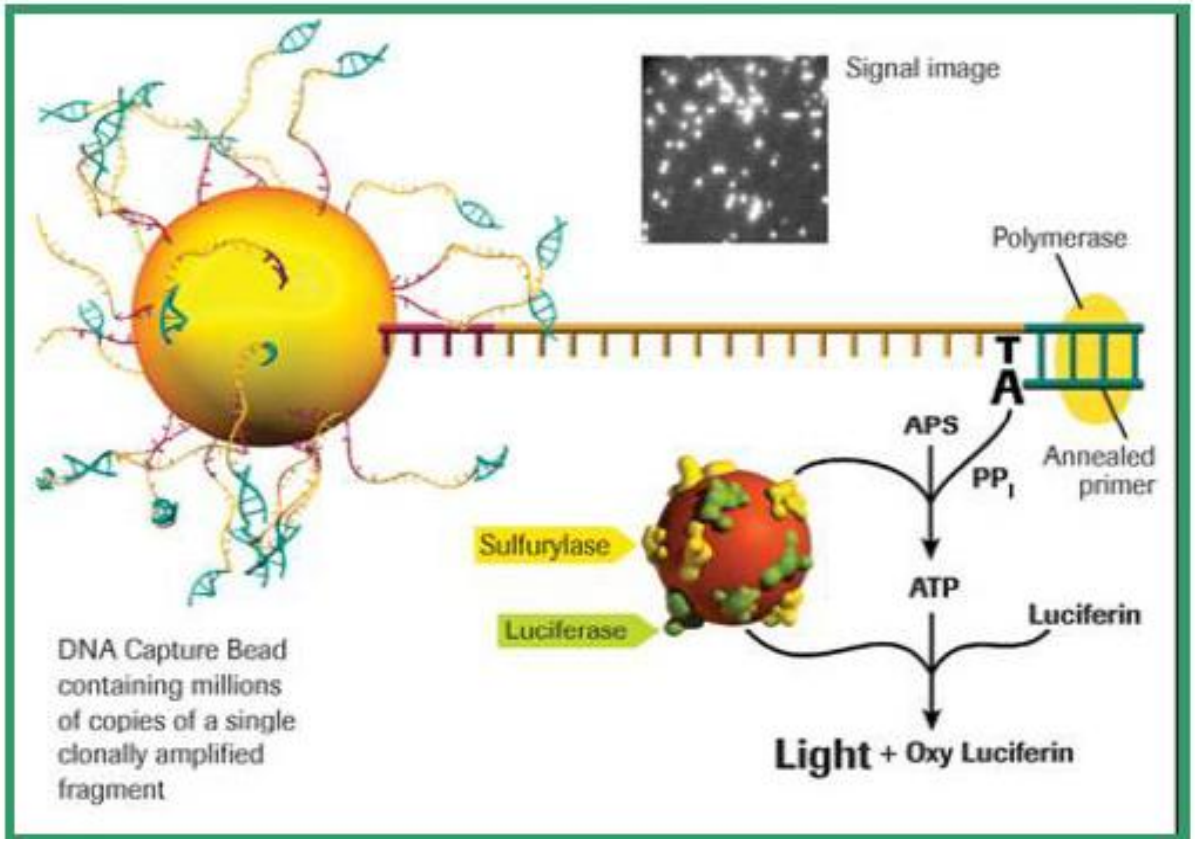
Hall, M.P., et al. (2012) ACS Chem Biol *7:1848-1857.*


Firefly Luciferase
Renilla Luciferase
NanoLuc™ Luciferase

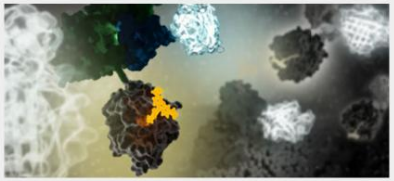
Biotechnological uses of luciferase

- Pyrosequencing (**454 sequencing**)
- In **PCA** to monitor **protein protein interactions**
- To test for anti-oxidants

- **As a reporter gene**
  - To assess **promoter strength and activity**
  - ***In vivo* imaging**
  - Cancer biology


Signal image
Polymerase
T
A
APS
PPi
Annealed primer
Sulfurylase
Luciferase
ATP
Luciferin
DNA Capture Bead containing millions of copies of a single clonally amplified fragment
Light + Oxy Luciferin

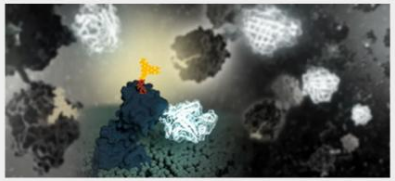
Explore NanoLuc® Luciferase



Protein:Protein Interactions

New ways to understand protein interactions, identify the partners involved, and decipher their biological significance.

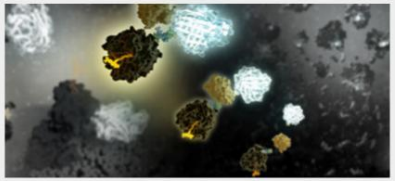
Protein:Protein Interactions



Target Engagement

Quantify the interaction between a molecule and a protein in real time, live cell assays.

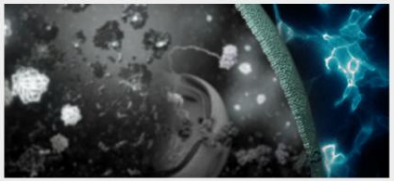
Target Engagement



Biosensors

BRET-based biosensors to detect signaling events inside and outside of the cell.

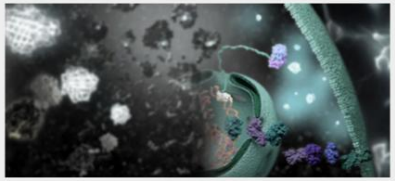
Biosensors



Imaging

Sensitive, bright bioluminescent imaging in cells and whole animals.

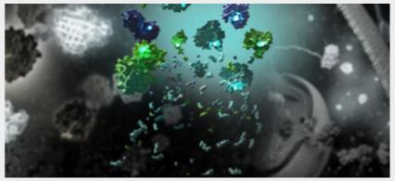
Imaging



Gene Regulation & Cell Signaling

Greater sensitivity and versatility for traditional gene regulation assays.

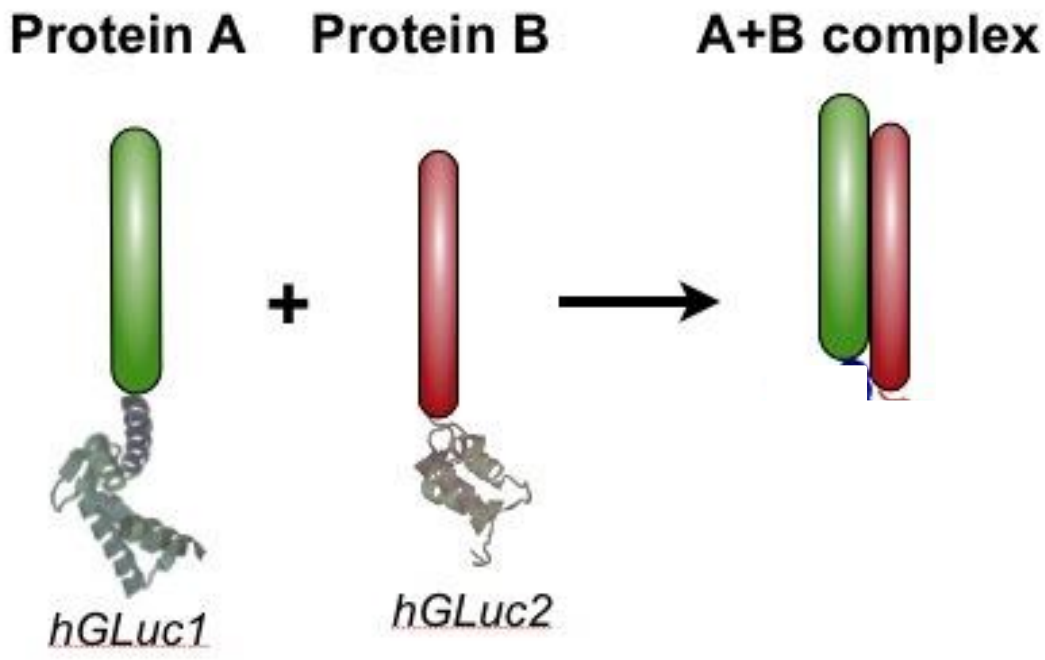
Gene Regulation & Cell Signaling

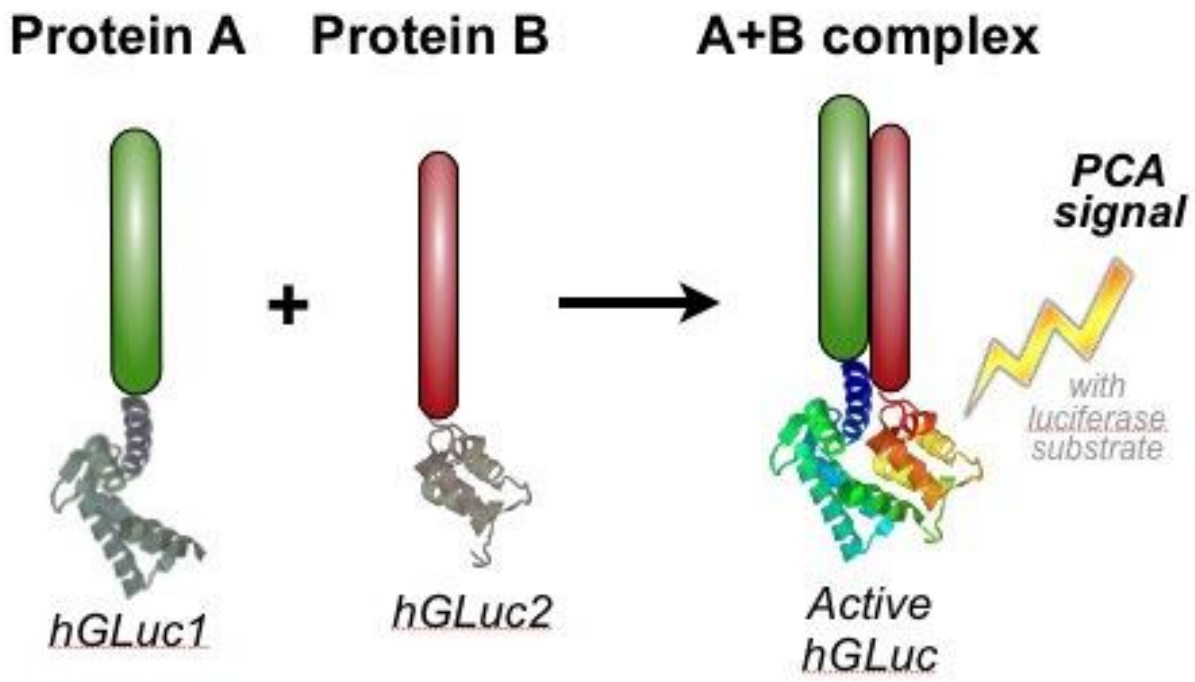


Protein Stability

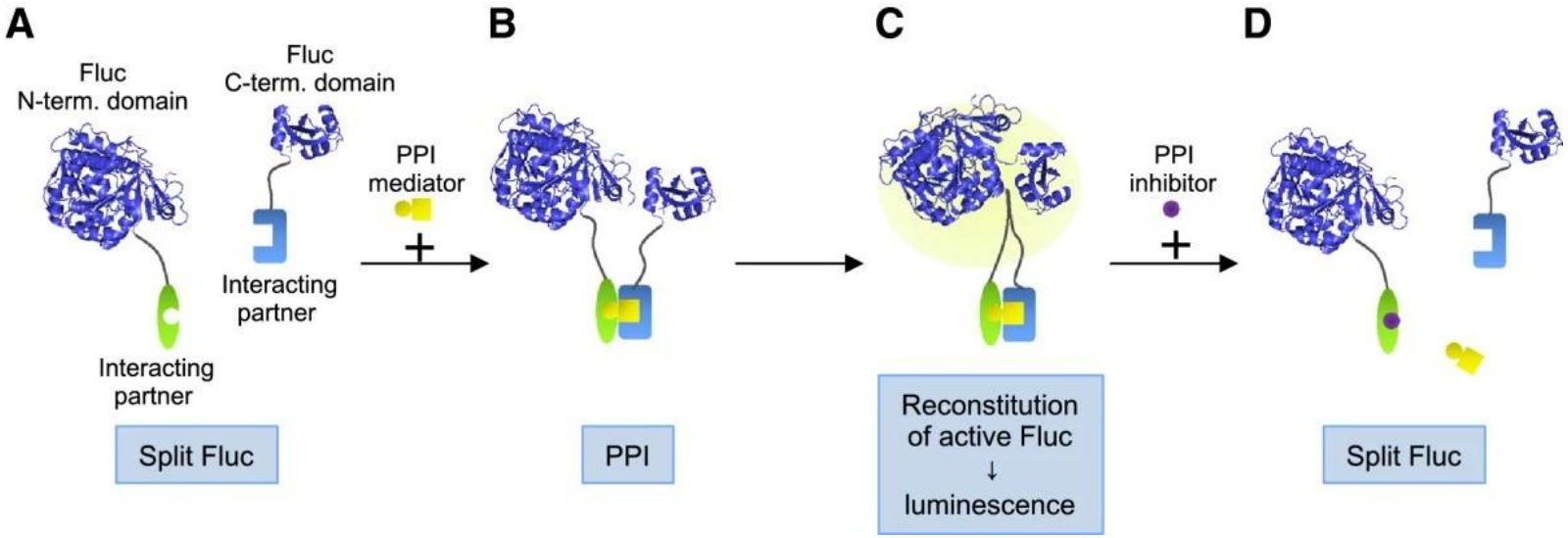
Monitor changes in intracellular protein abundance with NanoLuc® fusion proteins.

Protein Stability





The Principle of Protein-Fragment Complementation Assays (**PCA)**

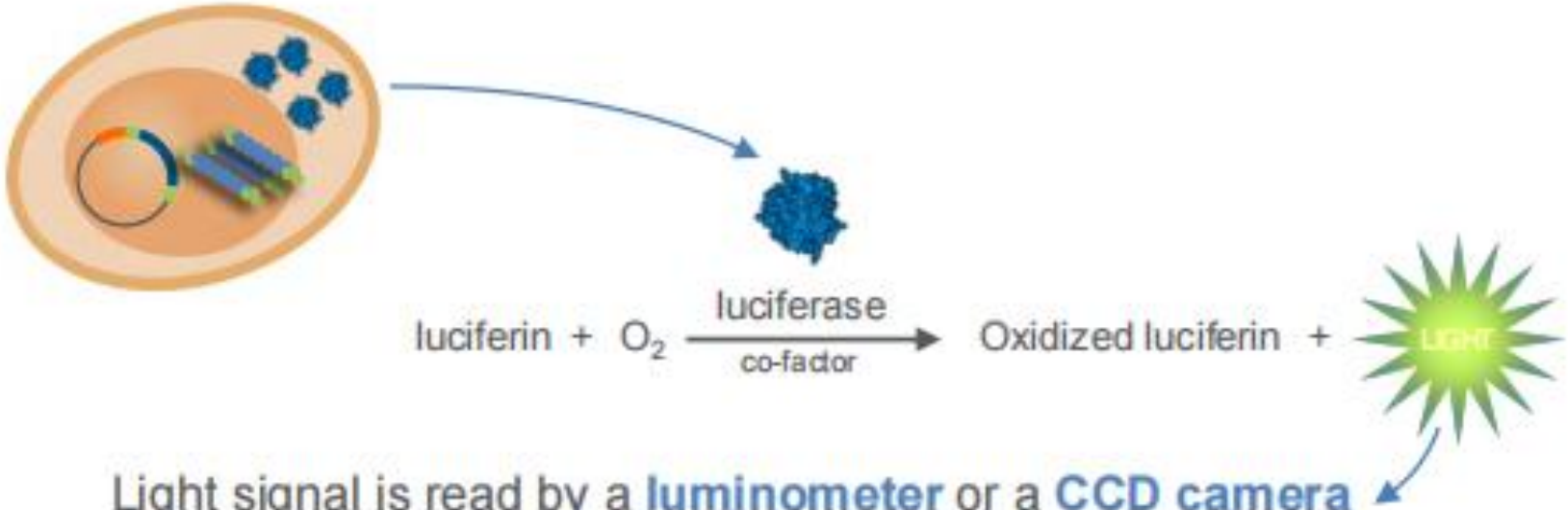


HT-monitoring of luciferase activity on 96/384 well plate luminometer

Bioluminescence In The Lab - Luciferase As A Reporter

Luciferase is the generic name for the class of enzyme

*(the substrate is generically referred to as **luciferin**)*



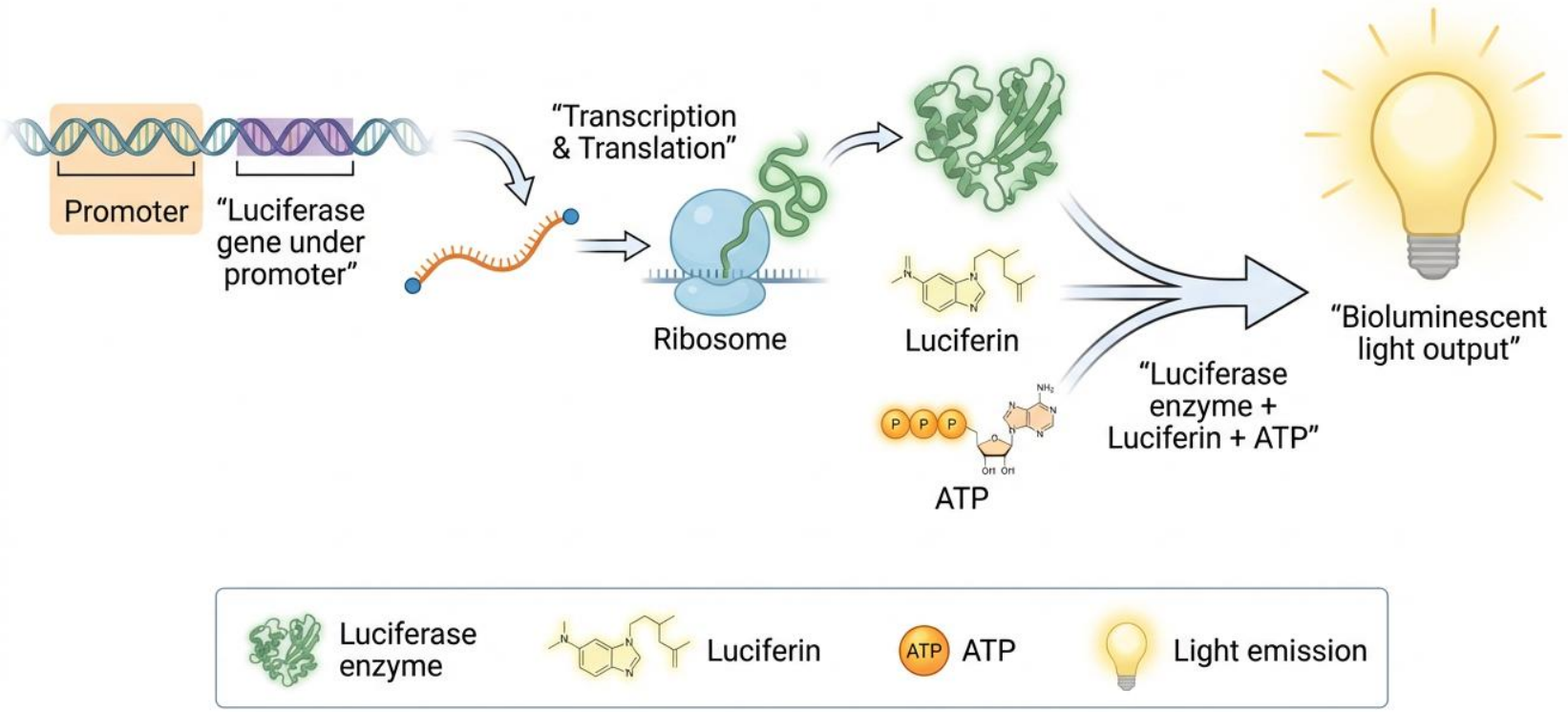
Light signal is read by a **luminometer** or a **CCD camera**

Lytic *-OR-* **Live** Assay

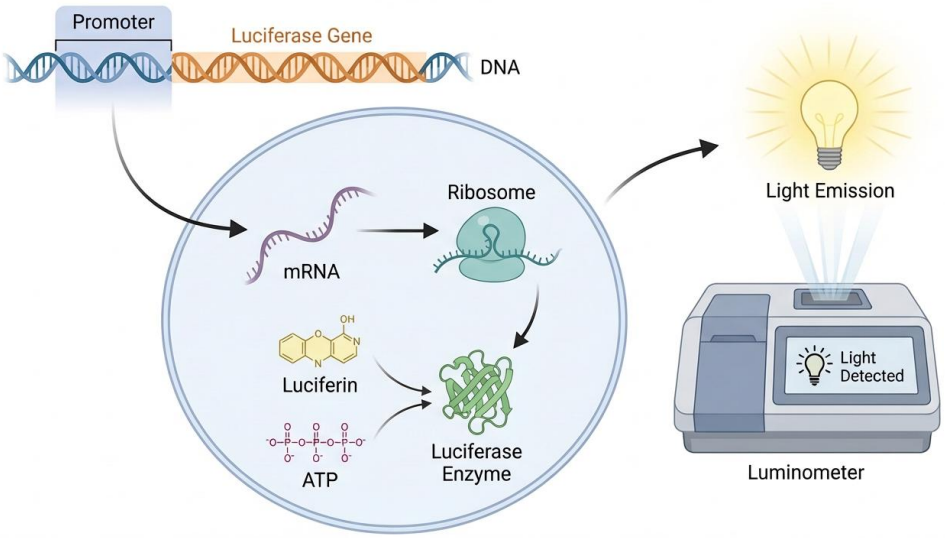
What is a Reporter?

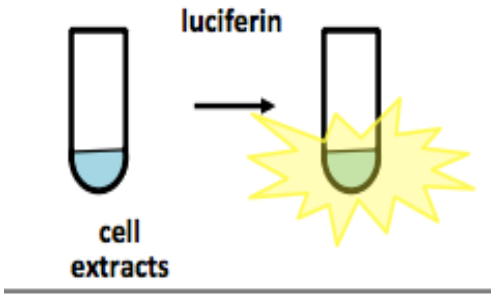
- ***Genetic reporters*** are ***indicators*** of ***gene expression*** *or* ***cellular events*** *coupled to gene expression*
- Reporters can mark ***any gene product***
  - **Transcriptional Fusion** – *reports on transcriptional and post-transcriptional regulatory inputs & events*
  - **Translational Fusion** – *reports on post-translational regulatory inputs & events*
- Reporters may be used in ***cells, tissues,*** *or* ***whole organisms***

Principle of Luciferase as a Gene Reporter

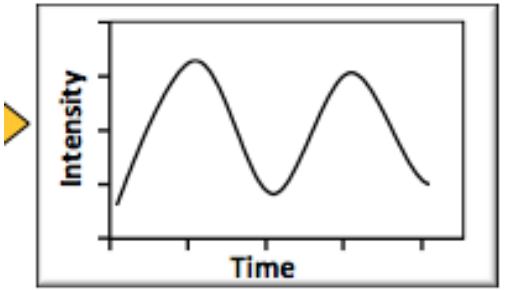


The luciferase reporter assay is commonly used as a tool to study
- gene expression - at the transcriptional level.





Real-time monitoring



It is widely used because it is

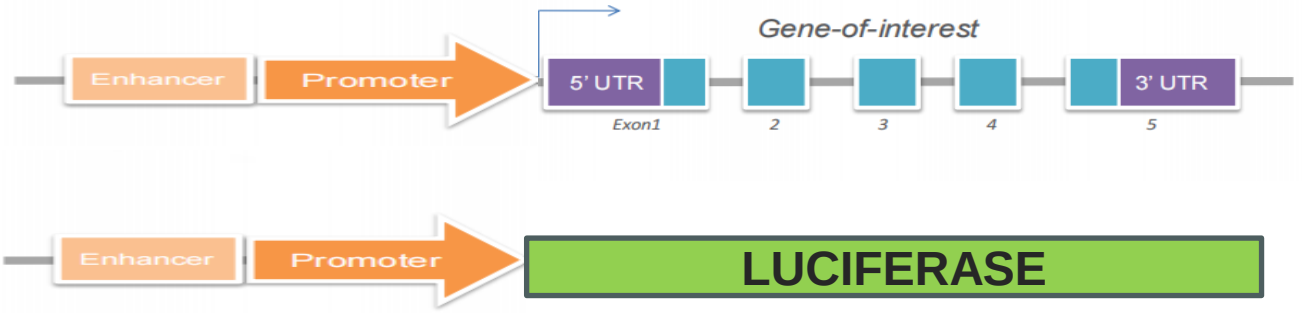
- **convenient,**
- **relatively inexpensive,**
- gives **quantitative measurements instantaneously**.

It has broad applications across various fields of cell and molecular biology – wherever you want to **measure** or **track** **expression of a cloned gene**.

Reporters Can Be Used For A Variety Of Applications

Gene expression:

- **Transcription and post-transcriptional regulation**
  - Promoters/response elements
  - Enhancers
  - 5'- and 3'-UTRs
  - Transcriptions factors
  - RNA binding proteins & miRNAs
- **Post-translational regulation**
  - Protein stability
  - Protein localization
  - Protein:protein interactions



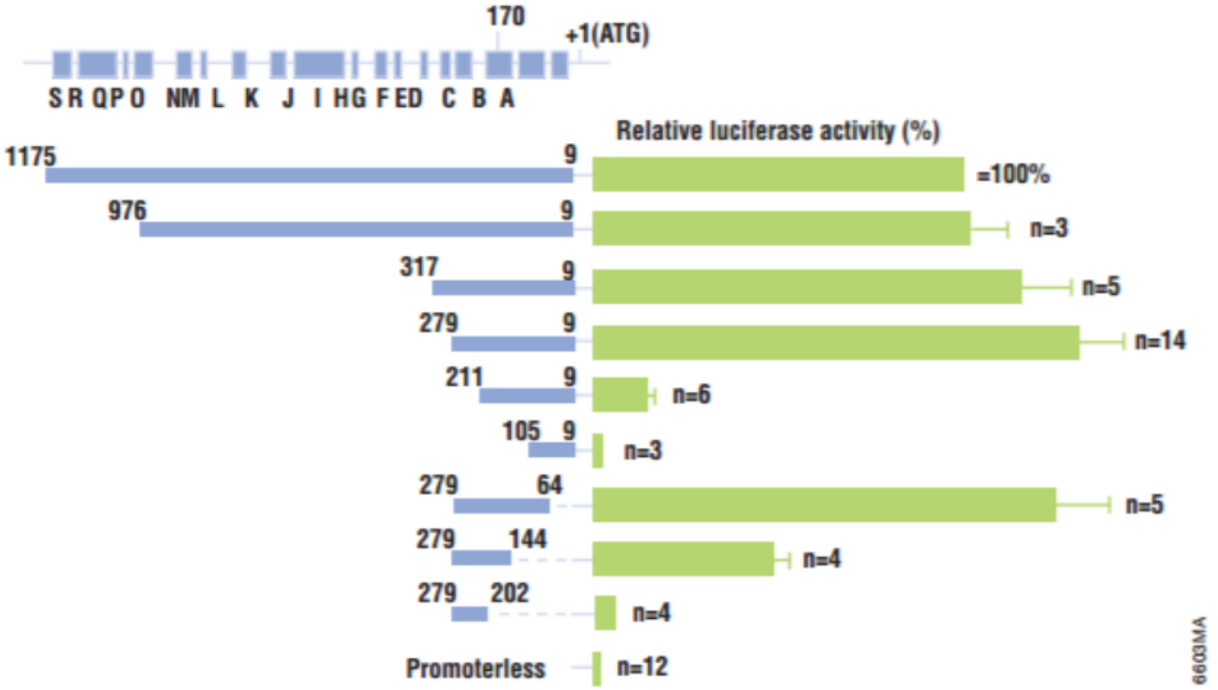
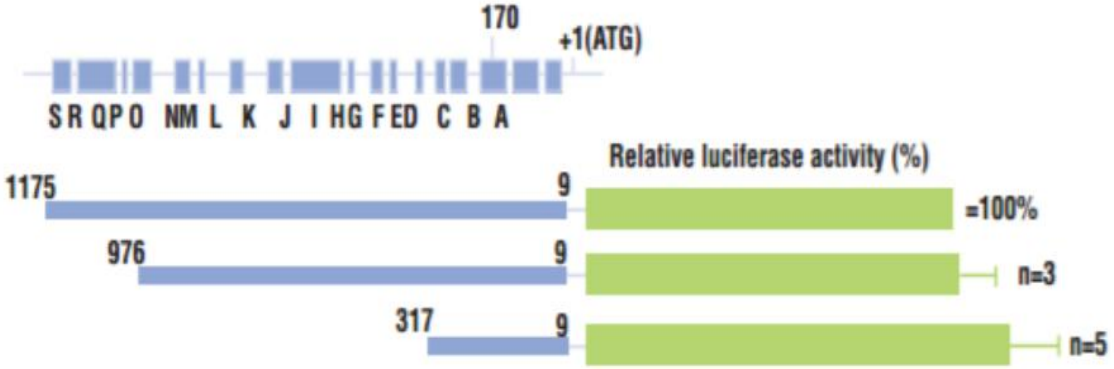


Figure 3. On the basis of observed luciferase activity, a promoter region of about 200 bp was identified that contained the necessary elements for adequate expression of the factor VIII-luciferase construct. Solid blue bars to the left indicate promoter regions that were placed upstream of the luciferase sequence. Green bars on the right indicate the level of luciferase activity relative to the intact sequence.

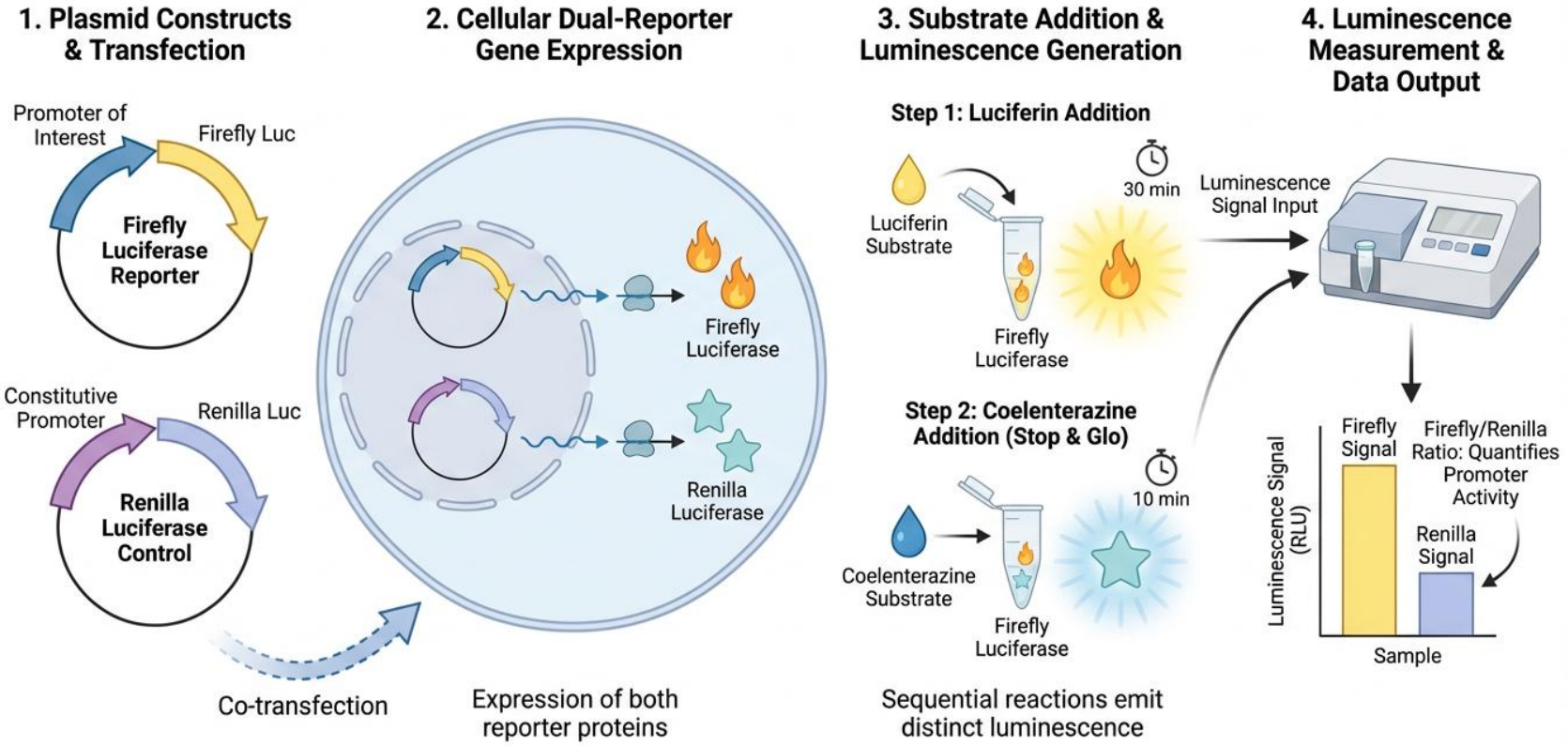


Normalization Assay Is An Important Control

Several parameters in a cell-based reporter experiment can cause variation or artifacts in reporter signal

- **Starting cell number** - *pipetting variation, problems with clumping/dispersion*
- **Transfection efficiency** - *related to cell density*
- **Ending cell number** - *cytotoxic effect of treatment; detached cells lost in media transfers or washing steps*

Schematic Workflow and Mechanism of the Dual-Luciferase Reporter Assay



Normalization Using A Co-reporter

Co-reporter, or normalization reporter:

- A second, **compatible reporter** gene is **co-transfected** with the primary reporter plasmid. Driven by a **"constitutive" promoter**
- Controls for **cell number** *AND* **transfection efficiency**. Can also serve as a control for **specificity of effect**
- Measured along with primary reporter using a **dual reporter assay**

Dual-Luciferase Reporter Assay System

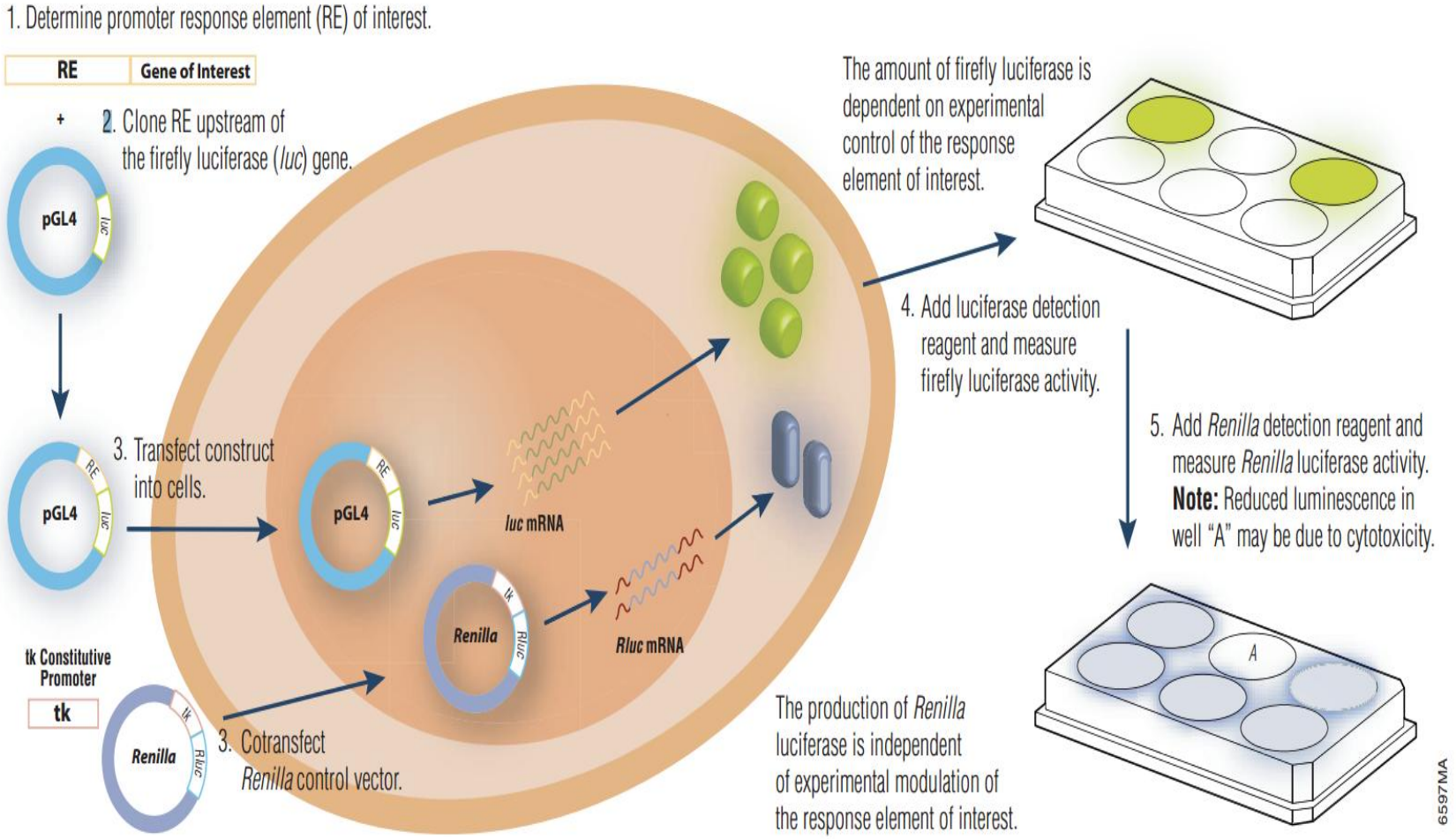


Figure 1. The DLR™ and Dual-Glo™ Assays provide rapid and convenient means for achieving greater control over the biological significance of reporter data by differentiating genetic responses of interest from nonrelevant influences in the experimental system.

Reporters Can Be Used For A Variety Of Applications

Gene expression:

- **Transcription and post-transcriptional regulation**
  - Promoters/response elements
  - Enhancers
  - 5'- and 3'-UTRs
  - Transcriptions factors
  - RNA binding proteins & miRNAs
- **Post-translational regulation**
  - Protein stability
  - Protein localization
  - Protein:protein interactions

Cellular Events:

- **Receptor activation/signaling**
  - Receptor ligands, agonists & antagonists
  - Nuclear receptors
- **Pathway analysis**
  - Defining pathways
  - Protein:protein interactions
- **Disease/Immune responses**
  - Cellular response to infection
  - Cellular response to therapy
  - Infectious agent replication/response to therapy

Translational Fusion To The Reporter Gene

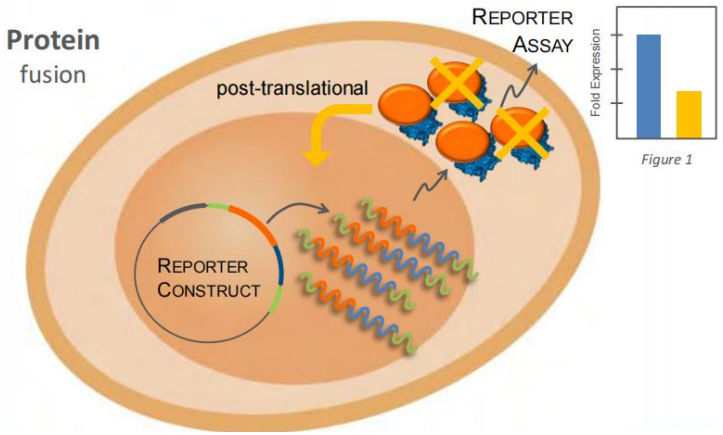
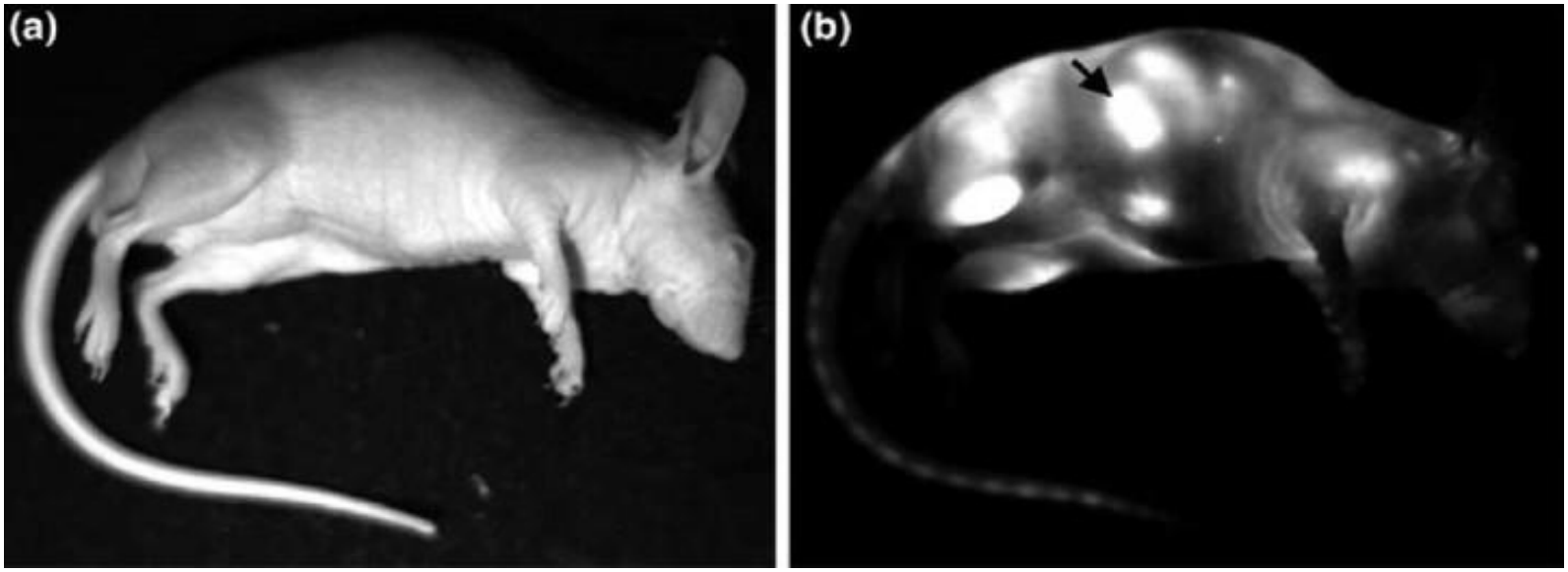


Figure 1

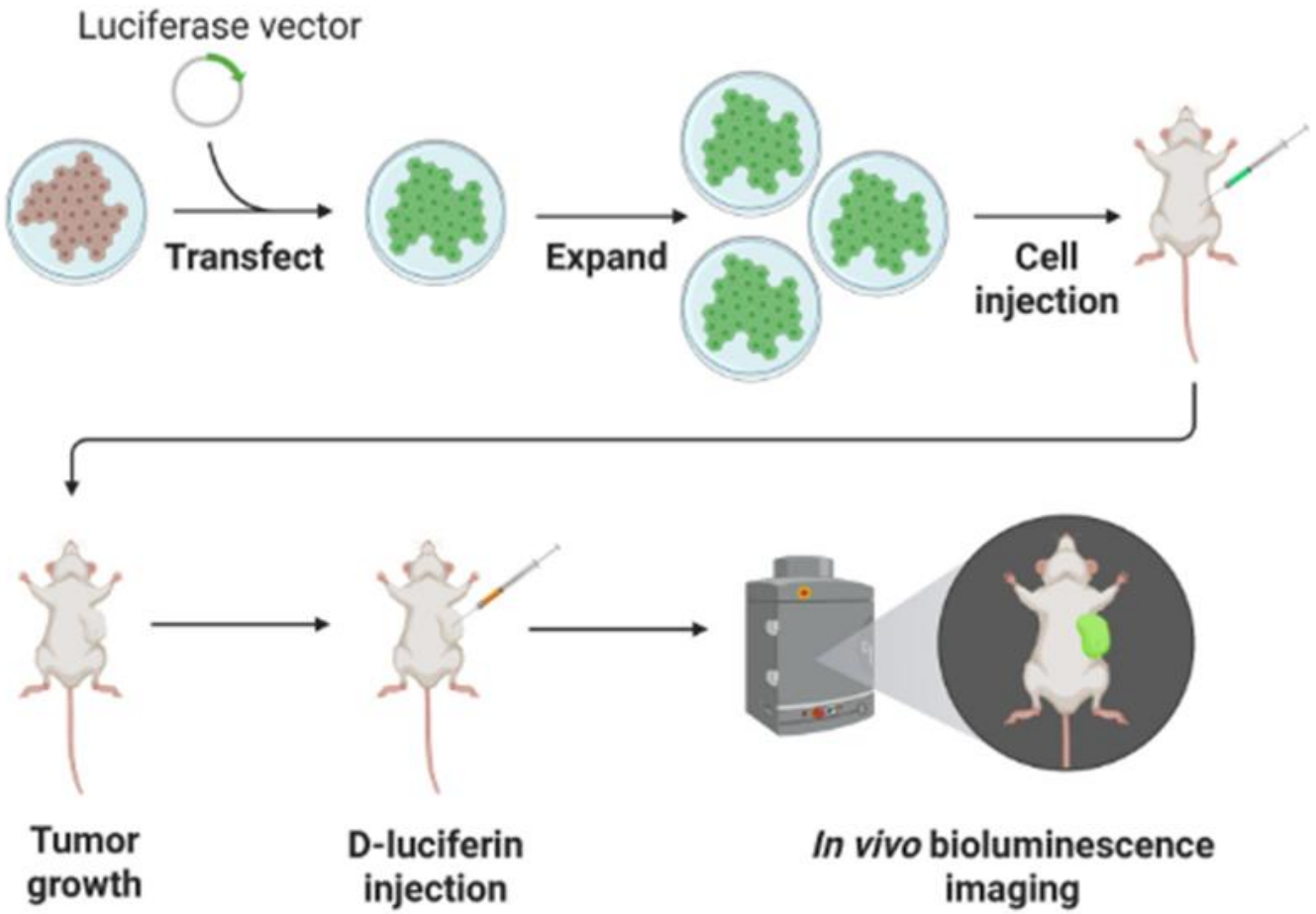
In vivo imaging of luciferase expression in mice

Since the luciferase gene can be stably transfected into cells under the control of most any promoter, **signal will not be lost following dilution by cell division**.

Luciferase assays in mammalian systems are particularly sensitive because they are **not subject to high background** as a result of tissue autofluorescence



Bioluminescence Imaging of Tumor Mass



Bioluminescent imaging is dependent on highly sensitive detectors.
Typically a **charged-coupled device (CCD) camera** is used to image luciferase signal in whole animals.

CCD cameras can be cooled to reduce thermal noise, and therefore background.

Additionally, CCD systems detect **the entire visible spectrum and near infrared wavelengths**, allowing them to detect the light that is not absorbed by mammalian tissues

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Contact us to lock in this rate and learn more about our other reagents for your *in vivo* imaging needs.

Quantity D-Luciferin	Price (DDP) VAT excluded
1 - 20 grams	€400/g
20+ grams	€360/g

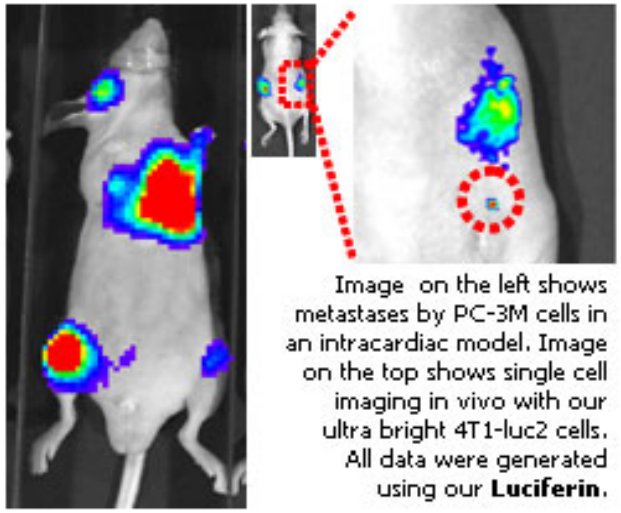
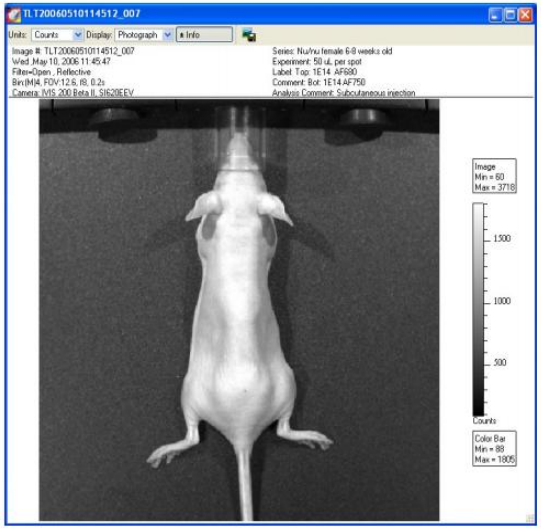
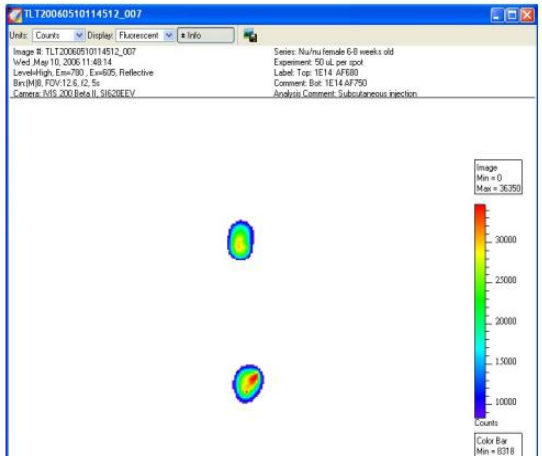


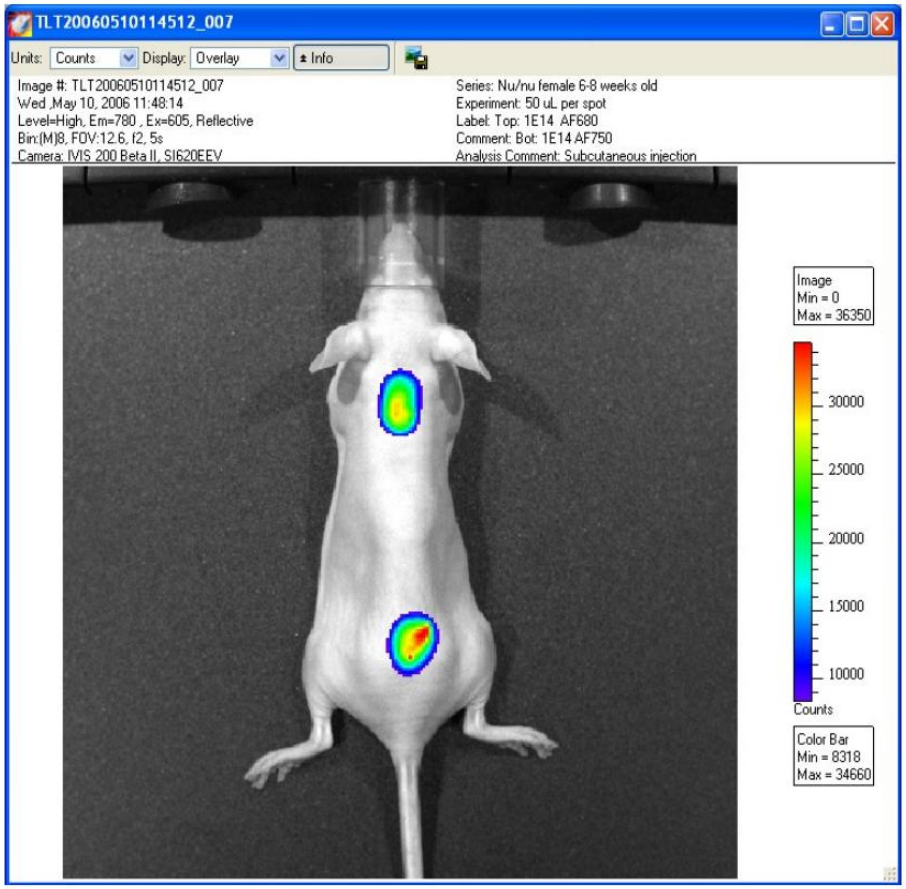
Image on the left shows metastases by PC-3M cells in an intracardiac model. Image on the top shows single cell imaging in vivo with our ultra bright 4T1-luc2 cells. All data were generated using our **Luciferin**.

For ordering, please contact:
Valérie Caldarola

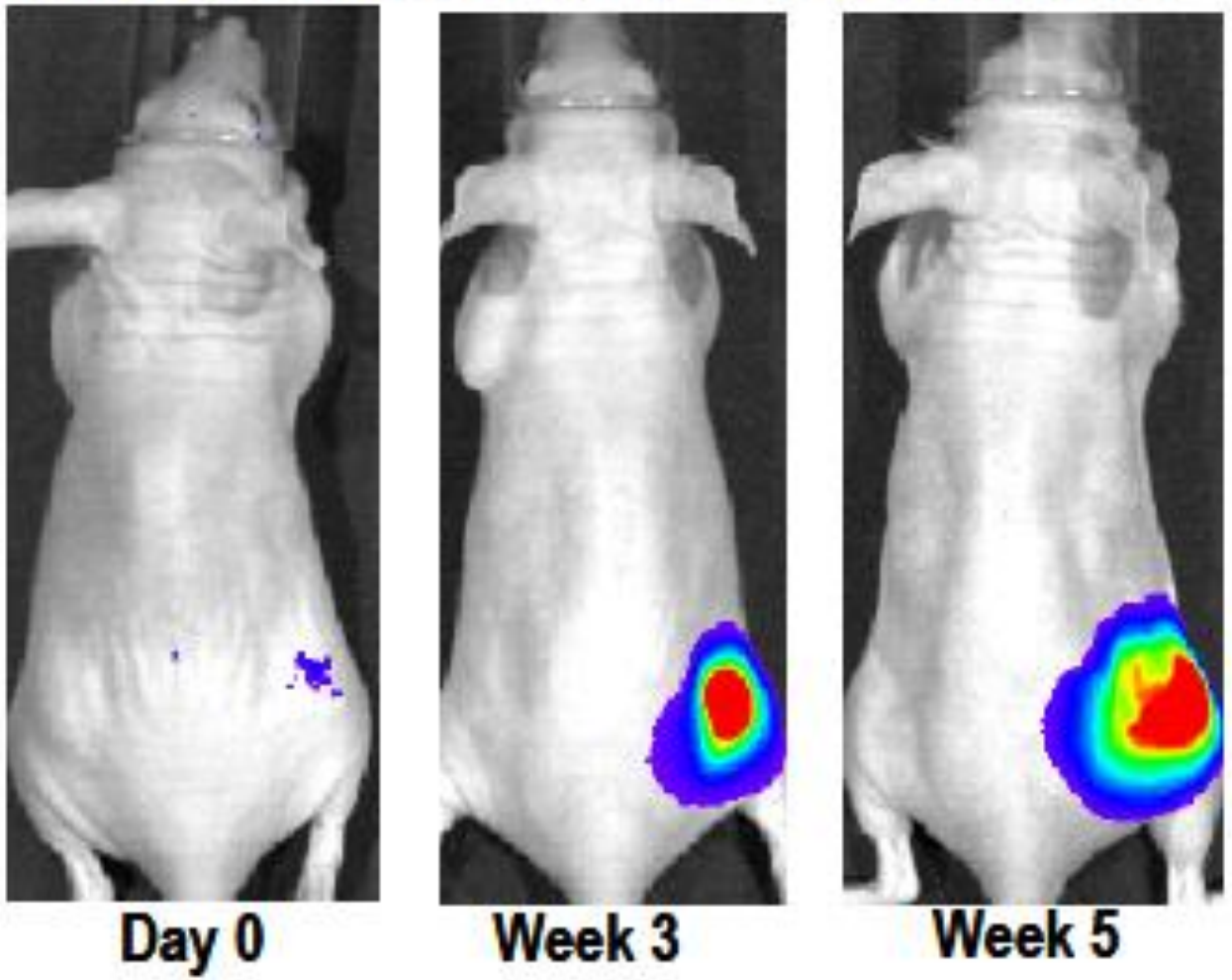
Standard Images are Composed of Two Images Photographic + Luminescent = Overlay



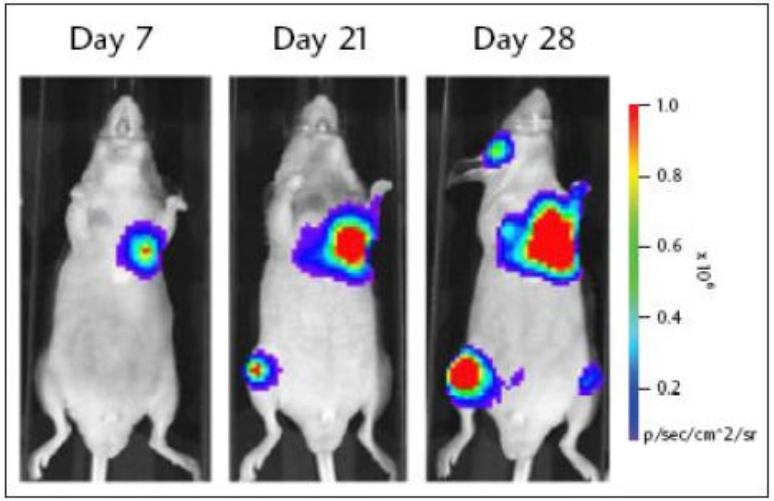




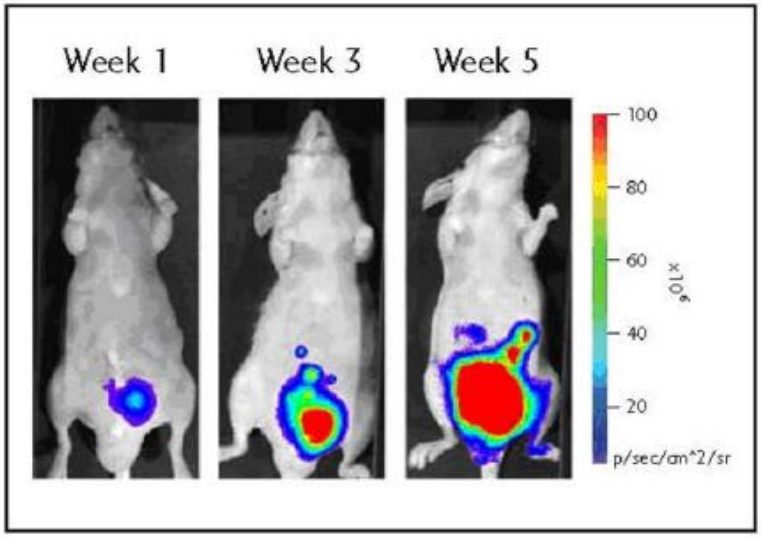
Bioware Ultra 4T1-luc2 Tumor Model



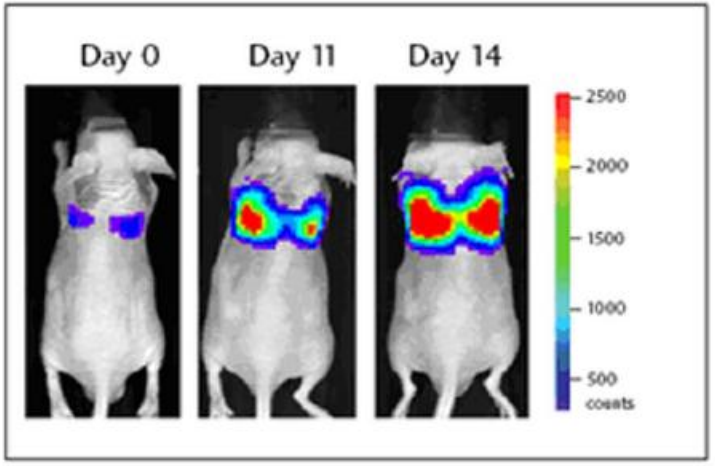
Bone metastasis



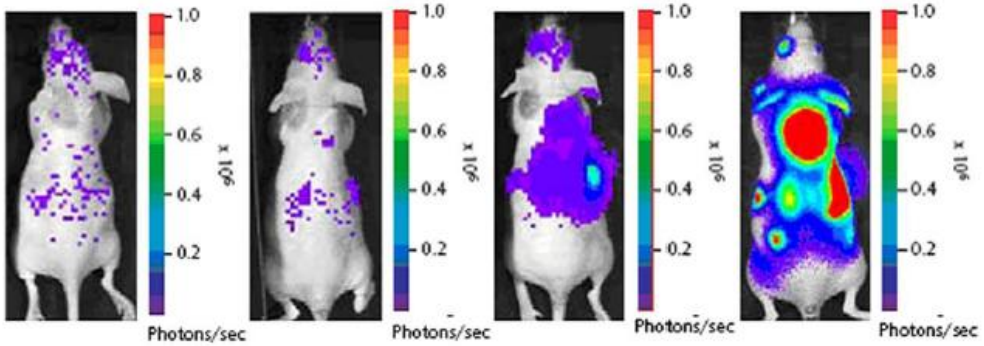
Prostate model

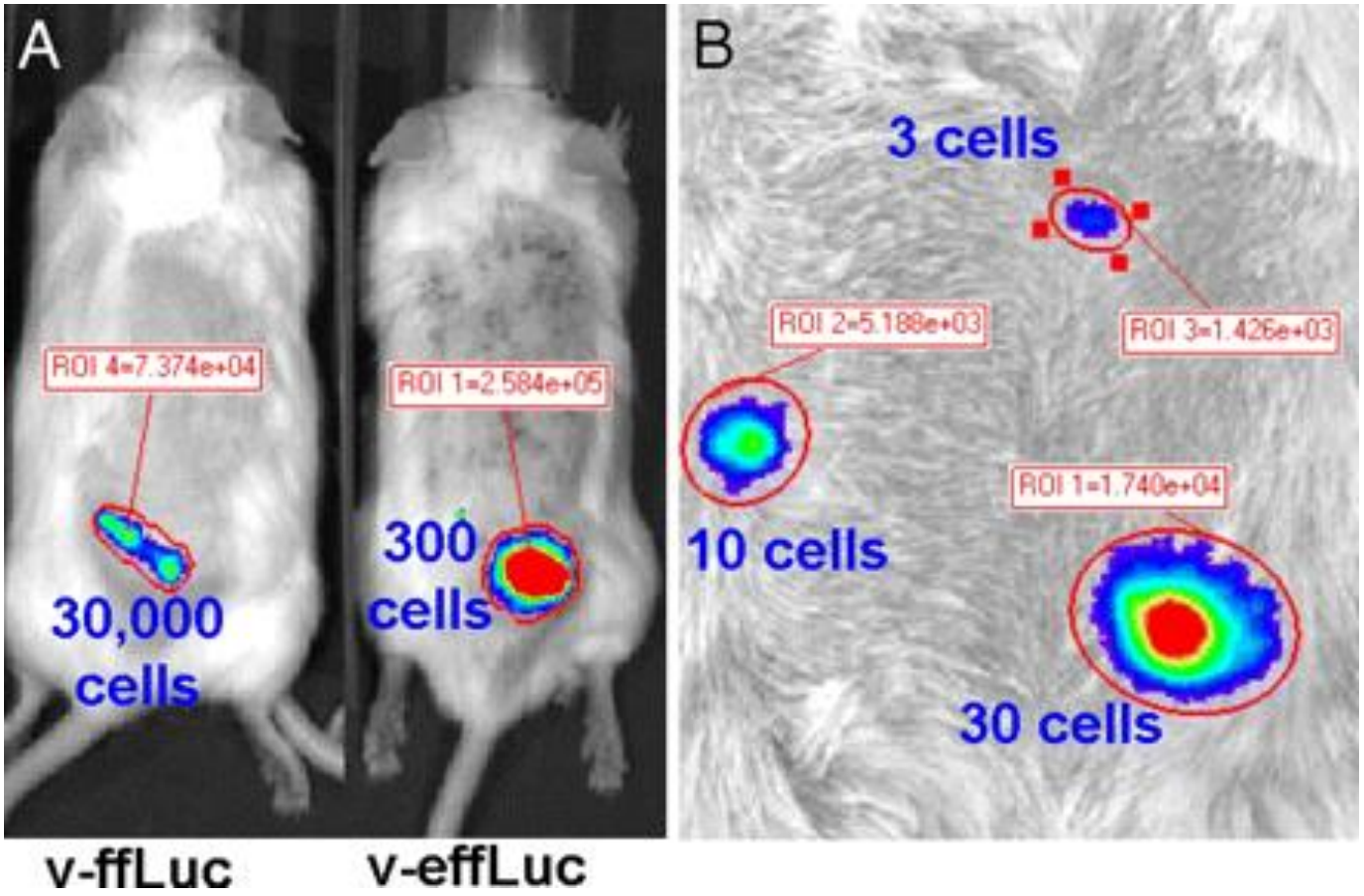


Melanoma

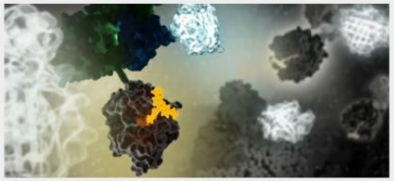


Breast cancer




A
ROI 4=7.374e+04
30,000
cells
ROI 1=2.584e+05
300
cells
v-ffLuc
v-effLuc
B
3 cells
ROI 2=5.188e+03
ROI 3=1.426e+03
10 cells
ROI 1=1.740e+04
30 cells

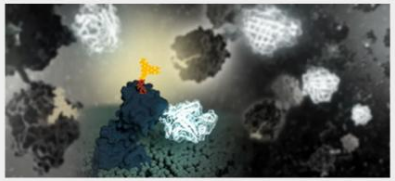
Explore NanoLuc® Luciferase



Protein:Protein Interactions

New ways to understand protein interactions, identify the partners involved, and decipher their biological significance.

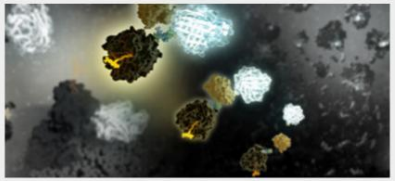
Protein:Protein Interactions



Target Engagement

Quantify the interaction between a molecule and a protein in real time, live cell assays.

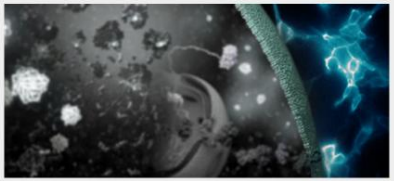
Target Engagement



Biosensors

BRET-based biosensors to detect signaling events inside and outside of the cell.

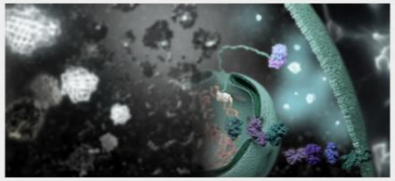
Biosensors



Imaging

Sensitive, bright bioluminescent imaging in cells and whole animals.

Imaging



Gene Regulation & Cell Signaling

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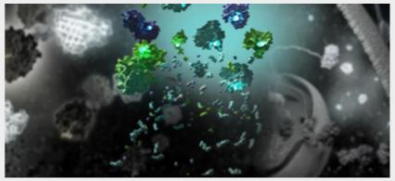
Gene Regulation & Cell Signaling



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Protein Stability