NanoLuc®: A Smaller, Brighter, and More Versatile Luciferase Reporter

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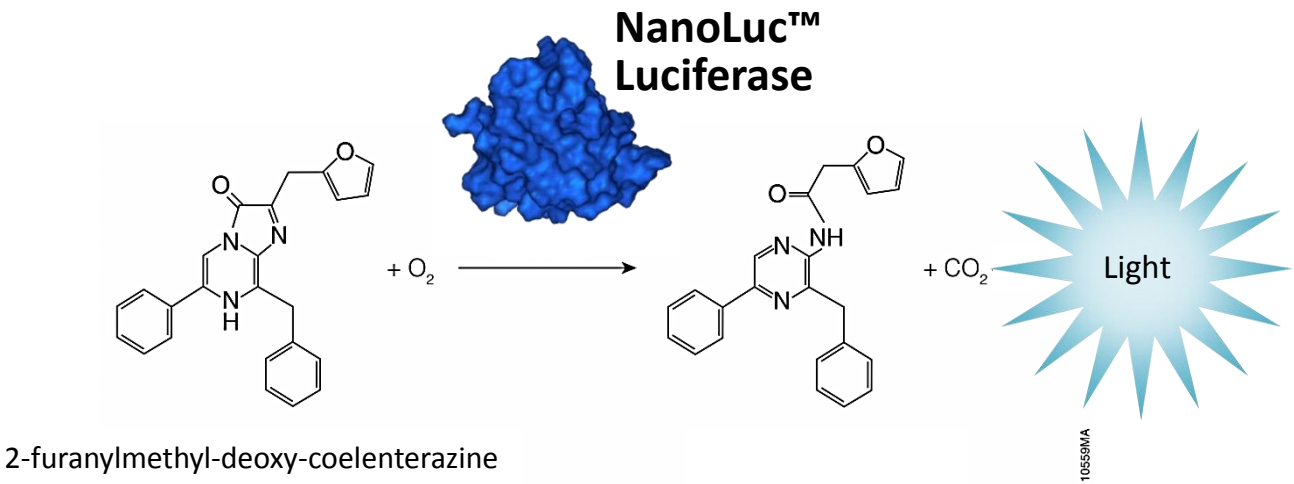
Promega Corporation

What is NanoLuc™ Luciferase?

NanoLuc™ (Nluc) is a 19.1 kDa, ATP-independent luciferase that utilizes a novel coelenterazine analog (furimazine) to produce high intensity, glow-type luminescence.

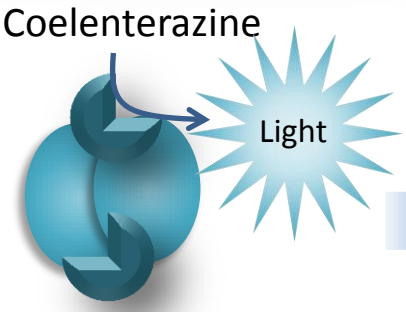


2-furanylmethyl-deoxy-coelenterazine

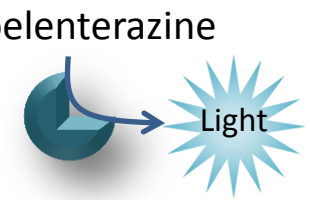
Furimazine **Furimamide**

Evolution of NanoLuc from ocean to lab bench

Oplophorus gracilirostris first cataloged in 1881



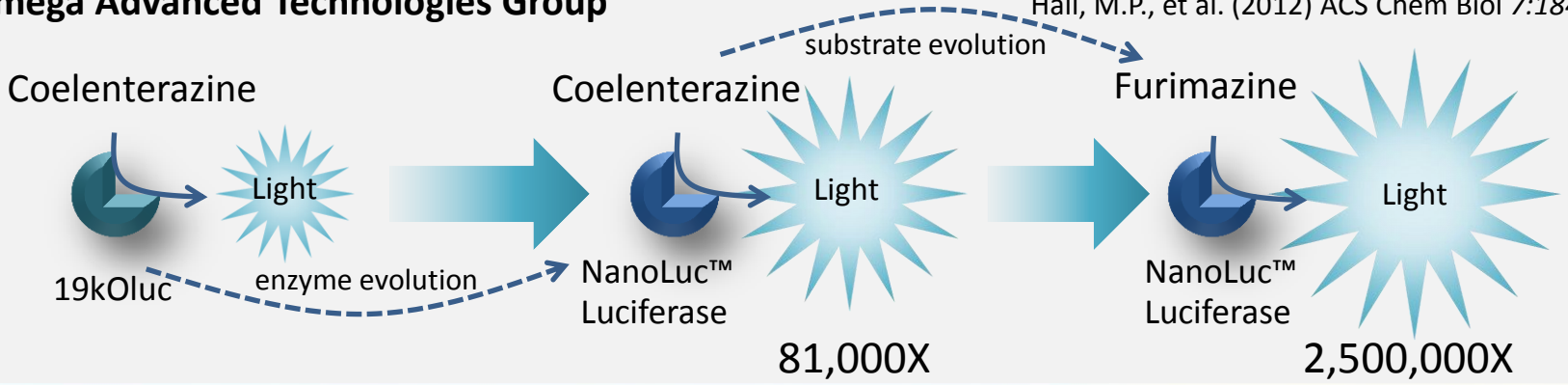
130kDa
Oplophorus luciferase
7X brighter than native
Renilla Luciferase
Shimomura, O., et al. (1978)



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

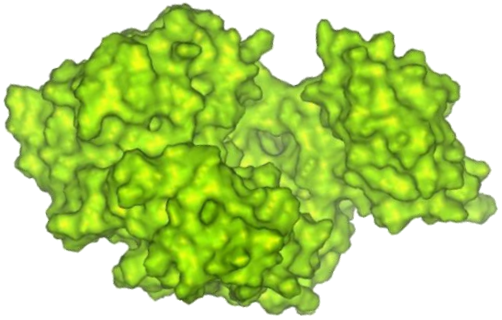
Promega Advanced Technologies Group

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

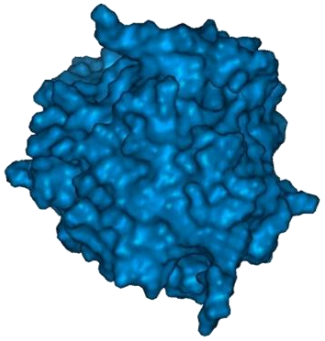


NanoLuc™ is very small

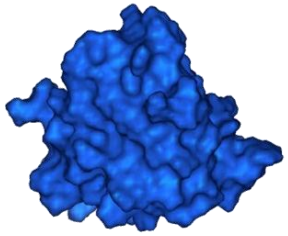
Firefly (Fluc)



Renilla (Rluc)

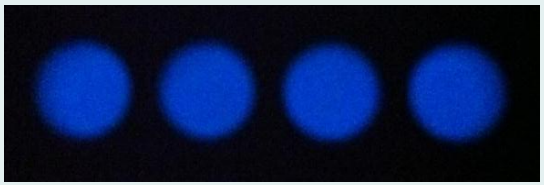


NanoLuc™ (Nluc)

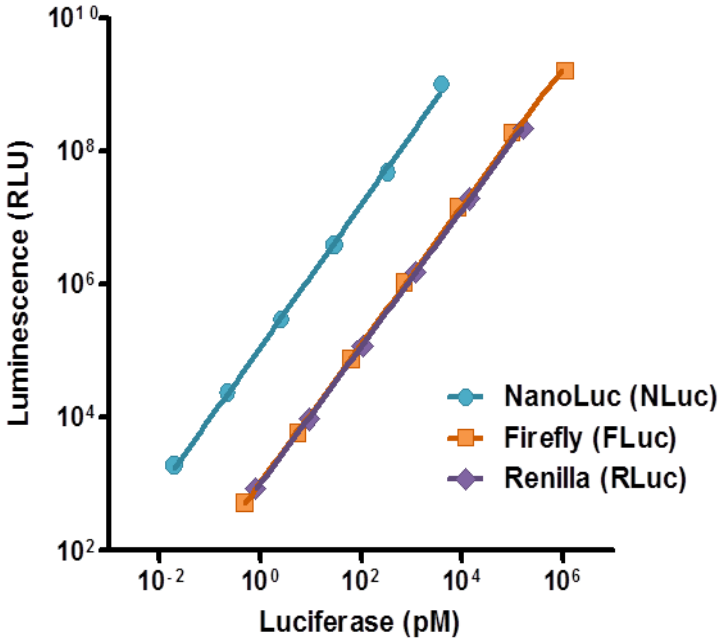


	Amino acids	M.W.	Mol. Vol. Å^3
Nluc	171	19.1	14
Rluc	312	36.0	32
Fluc	550	60.6	44

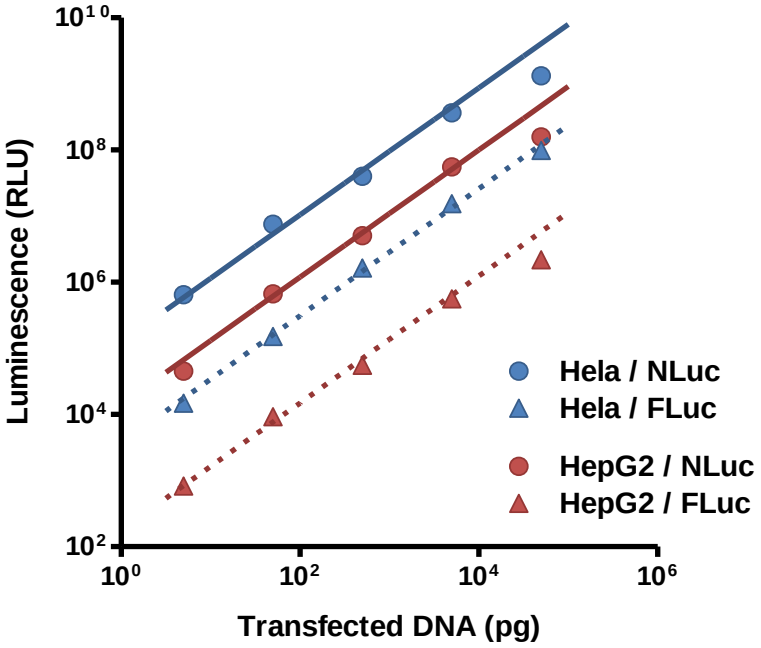
NanoLuc is very bright



Living HEK293 cells in 96-well plate (50,000 cells per well). Imaged by a hand-held iPhone



Purified enzyme



Expression in mammalian cells

NanoLuc™ has excellent physical properties

Thermal stable enzyme

- Retains activity following 30 min incubation at 55 °C
  - Melting temps: Nluc, 58 °C; Fluc, 31 °C

Active over broad pH range

- Fully active between pH 7-9
- Retains significant activity at pH 5-7
- Fluc: sharp decrease in activity below pH = 8

Monomeric enzyme

- Facilitates use as transcriptional reporter or fusion partner

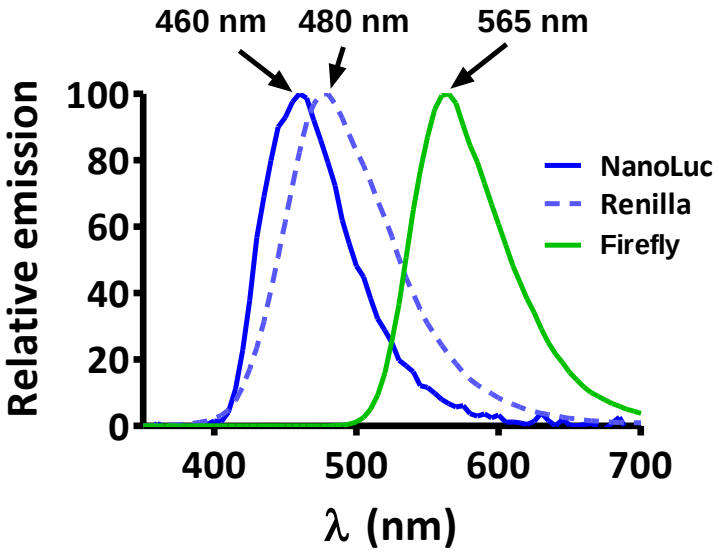
No post-translational modifications detected in mammalian cells

No disulfide bonds

- Supports high levels of activity inside living cells

Uniform distribution in cells

- No apparent compartmental bias in the absence of targeting sequences



unfused NLuc
Immunofluorescence

Nano-Glo™ Luciferase Assay Reagent

Nano-Glo™ Luciferase Assay Reagent:

Furimazine

- Provides maximal brightness

Glow kinetics (no flash reaction)

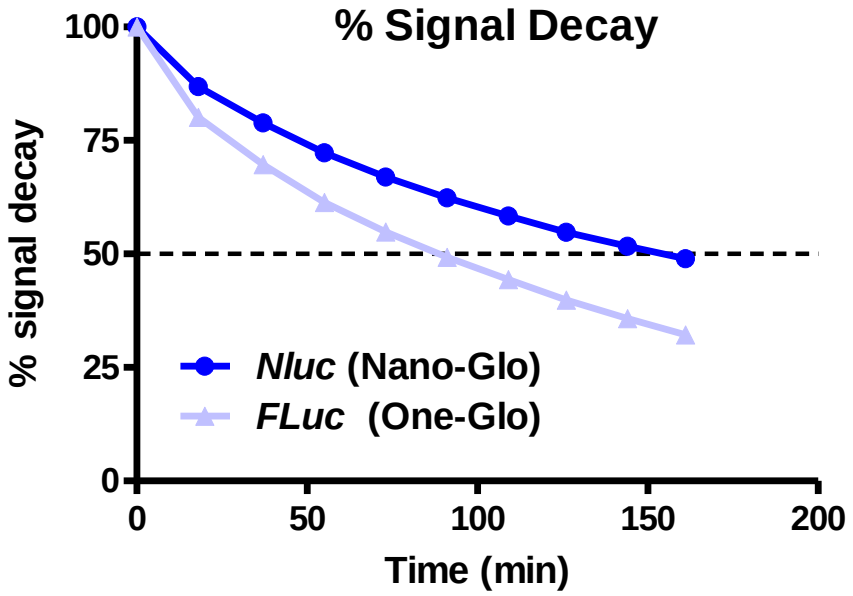
- Half-life routinely >2 hour at room temperature

Low autoluminescence background

- Enhances assay sensitivity

Stable reconstituted reagent:

- ~10% decrease in activity over 8 hrs at RT



Add-Mix-Measure format like

- ONE-Glo™ Luciferase Assay System
- Bright-Glo™ Luciferase Assay System
- Steady-Glo® Luciferase Assay System
- *Renilla*-Glo™ Luciferase Assay System

Reduced false hits with NanoLuc® Luciferase in HTS

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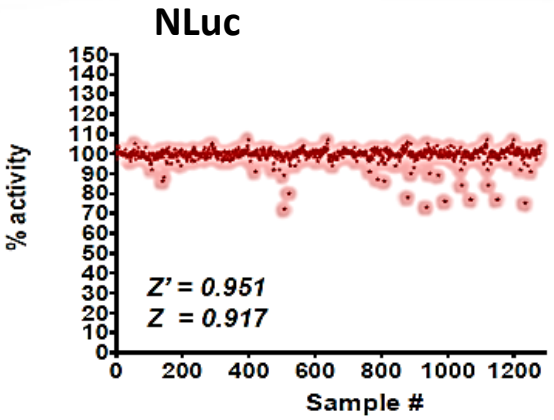
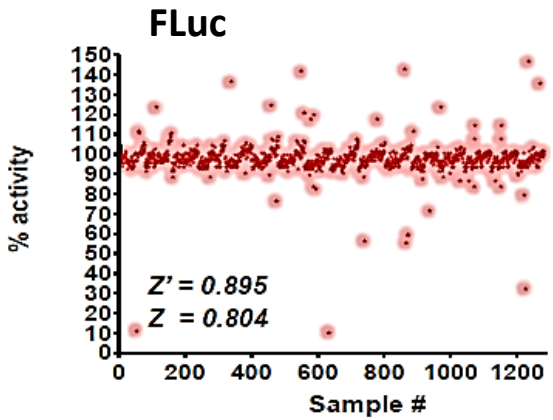
LOPAC library (Sigma)

- *Library of Pharmaceutically Active Compounds*
- 1280 compounds
- Small organic ligands w/ well documented pharmacological activities
- Used to screen for non-specific luciferase activity modulators

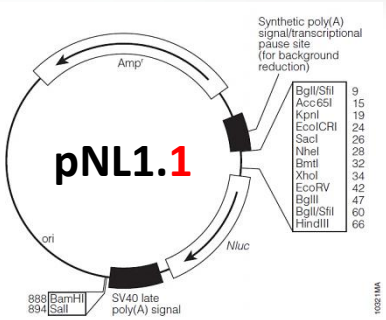


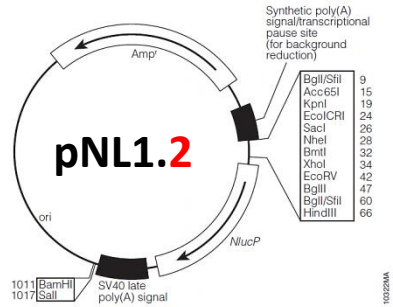


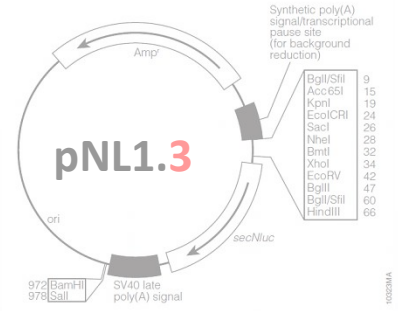
		Level of inhibition			
		≥ 10%	≥ 20%	≥ 30%	≥ 50%
% of library compounds	NanoLuc	1.2%	0.5%	-	-
	Firefly	1.9%	0.7%	0.5%	0.3%

Experimental details: LOPAC library members at 10 µM final concentration; incubation with purified NanoLuc or firefly luciferase for 2 min.; Fluc detection using ONE-Glo™ Luciferase Assay..

3 Varieties of NanoLuc® Luciferase for you







Intracellular Formats

Nluc (513 bp)

Protein destabilization domain

NlucP (636 bp)

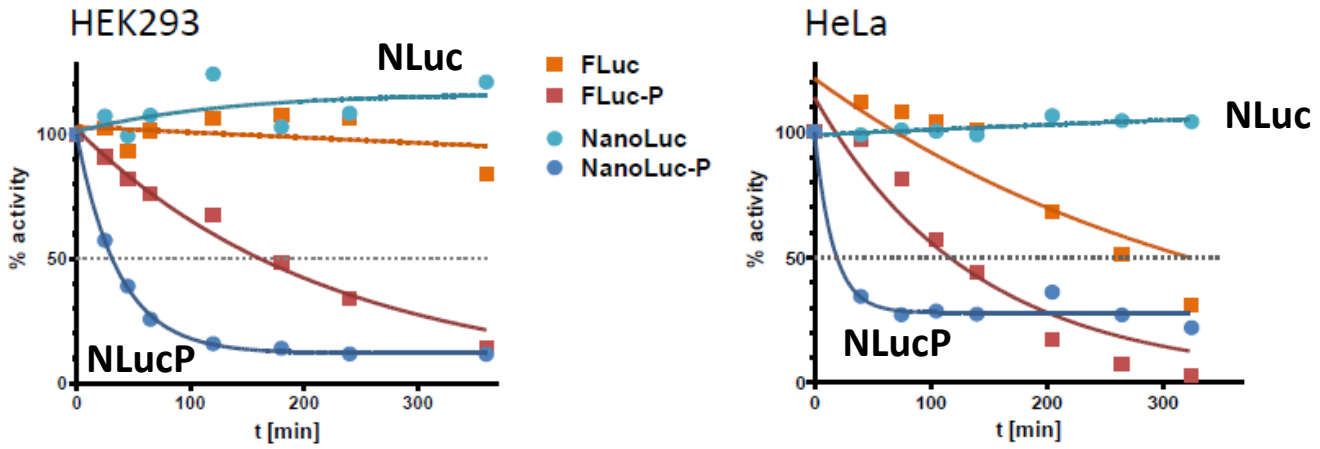
Secretion Format

secNluc (597 bp)

Secretion signal

Intracellular stability of NanoLuc™ & Firefly

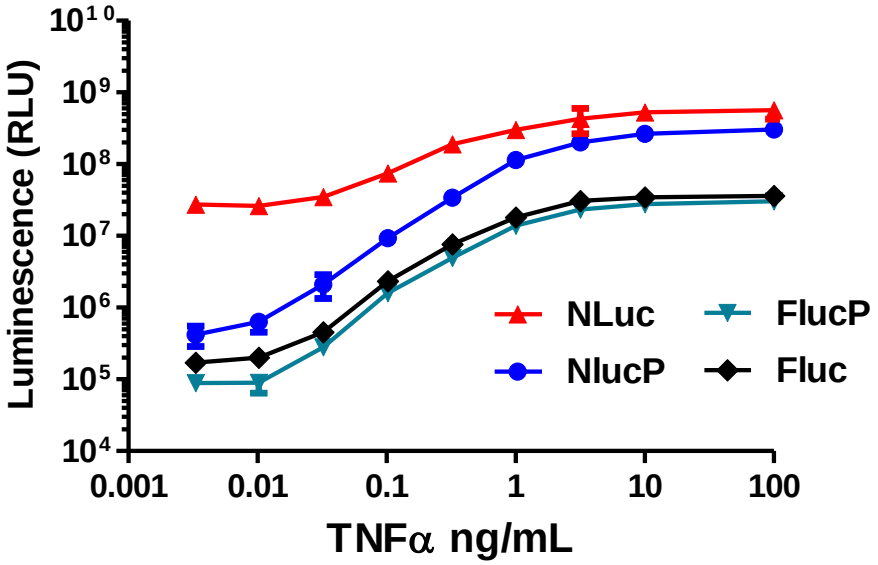
New protein synthesis blocked by addition of cycloheximide



Cell line	FLuc	FLucP	NLuc	NLucP
HEK-293	>6 h	2.0 ± 0.4 h	**>6 h**	**18 ± 11 min**
HeLa	3.8 ± 1.3 h	1.4 ± 0.2 h	**>6 h**	**20 ± 6 min**
U2OS (n=1)	>6 h	2.8 h	**>6 h**	**36 min**

Relative protein stability in cells: NlucP < FlucP < Fluc < Nluc

NlucP gives the greatest dynamic response



Experimental details: transient transfection of HEK293 cells with NF-κB inducible constructs. rhTNFα treatment for 5 hours.

Brightness

Nluc > NlucP > Fluc > FlucP

(18 experiments)

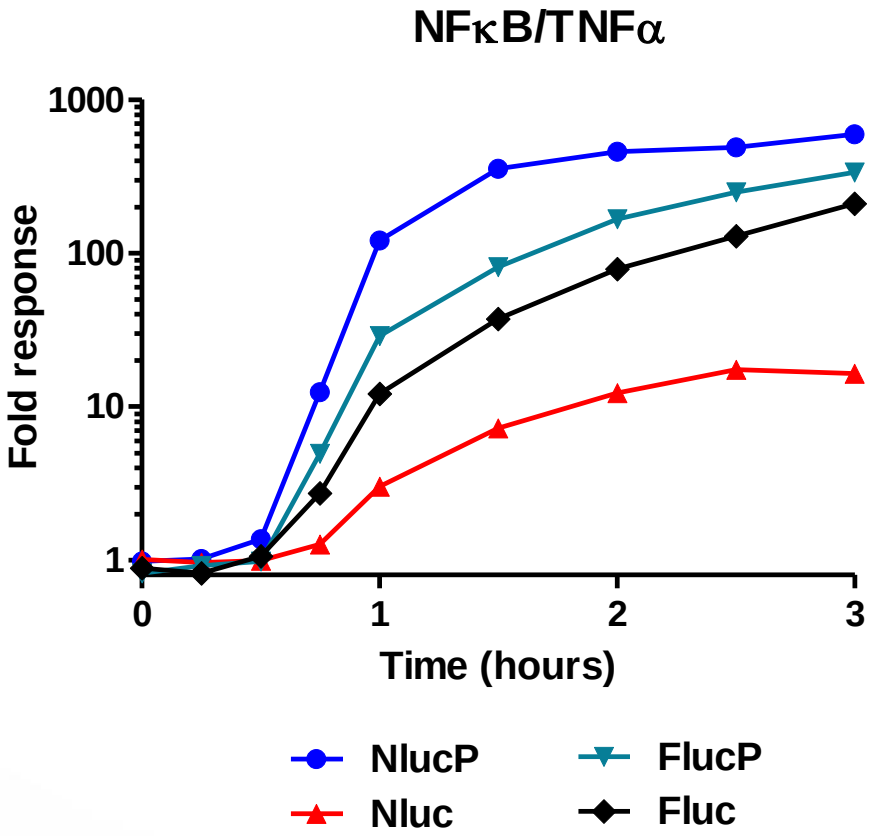
NLuc 13-236 fold brighter than Fluc (79 fold avg.)

NlucP 2-27 fold brighter than FLucP (10 fold avg.)

Nluc 10-78 fold brighter than NlucP (34 fold avg.)

→Very similar pharmacology/EC50s

NlucP responds earliest to stimuli



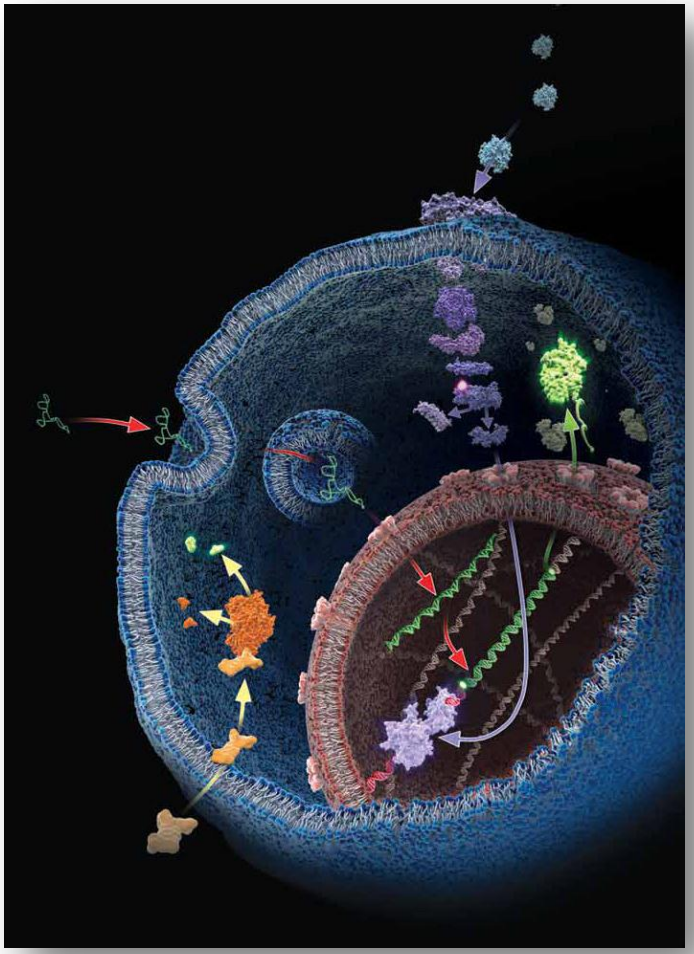
Relative Response

NlucP > FlucP > Fluc > Nluc

Experimental details: transient transfection of HEK293 cells with NFκB inducible constructs; addition of 100 ng/ml rhTNFα at time zero.

Summary: NanoLuc Luciferase as an intracellular reporter

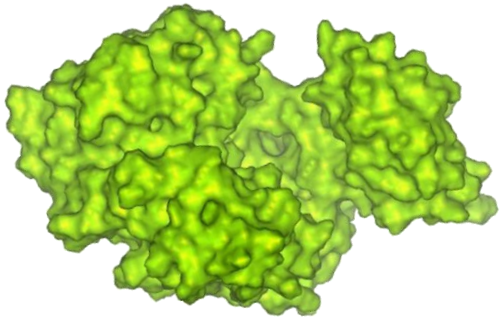
- ✓ NlucP for a faster response
- ✓ NlucP for greatest dynamic range
- ✓ NlucP for measuring weak responses
- ✓ Nluc where maximum brightness is needed.

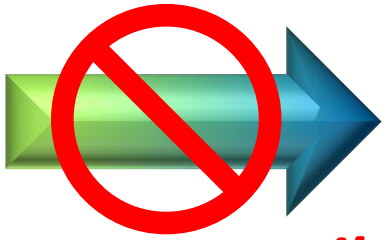


Should I switch from Firefly to NanoLuc™ Luciferase?

Promega

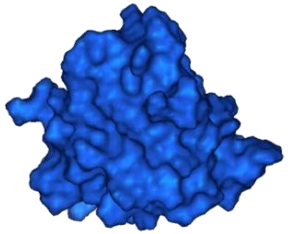
Firefly (Fluc)





Not necessarily

NanoLuc™ (Nluc)



Does it allow you to do your work?
Do you plan to do work in vivo?

Firefly is a great reporter

- ✓ Excellent signal:background
- ✓ Excellent dynamic range

We just released new response element signaling pathway detection pGL4 vectors:

ARE	HSE	ISRE	STAT5	SRE
p53	HRE	SIE	NFAT	SRF
ATF6	XRE	SBE	CRE	
MRE	AP1	TCF-LEF	NF-κB	

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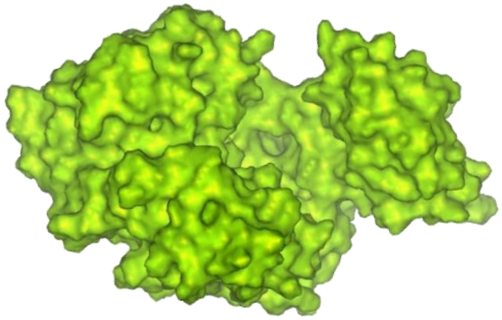
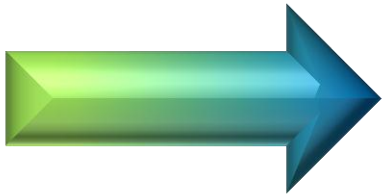
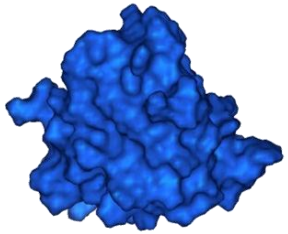
Firefly (Fluc)



Yes, if …



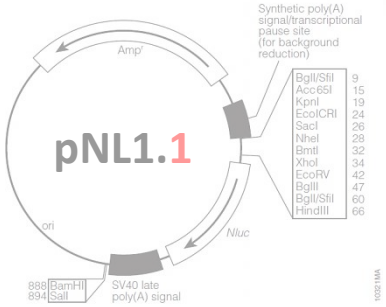
NanoLuc™ (Nluc)

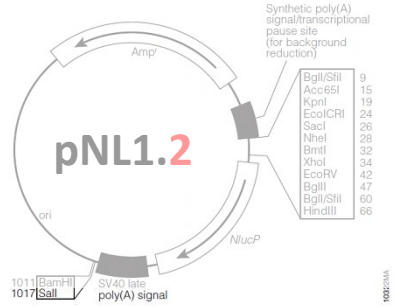


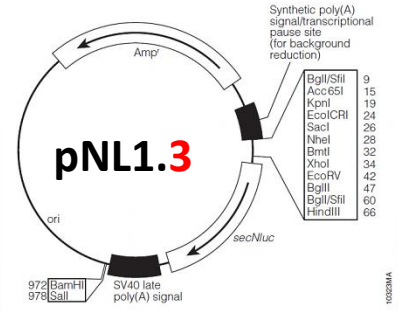
- ✓ **Transfection efficiency limits you to easy-to-transfect cell lines**
- ✓ **Signals are too weak to move to 96-well plates**
- ✓ **FLuc is just too big**

- The increased brightness could allow a subtle signal become a reliable signal.
- The small size could allow gene replacement with minimal impact, especially in viral constructs

3 Varieties of NanoLuc™ Luciferase for you







Intracellular Formats

Nluc (513 bp)

Protein destabilization domain

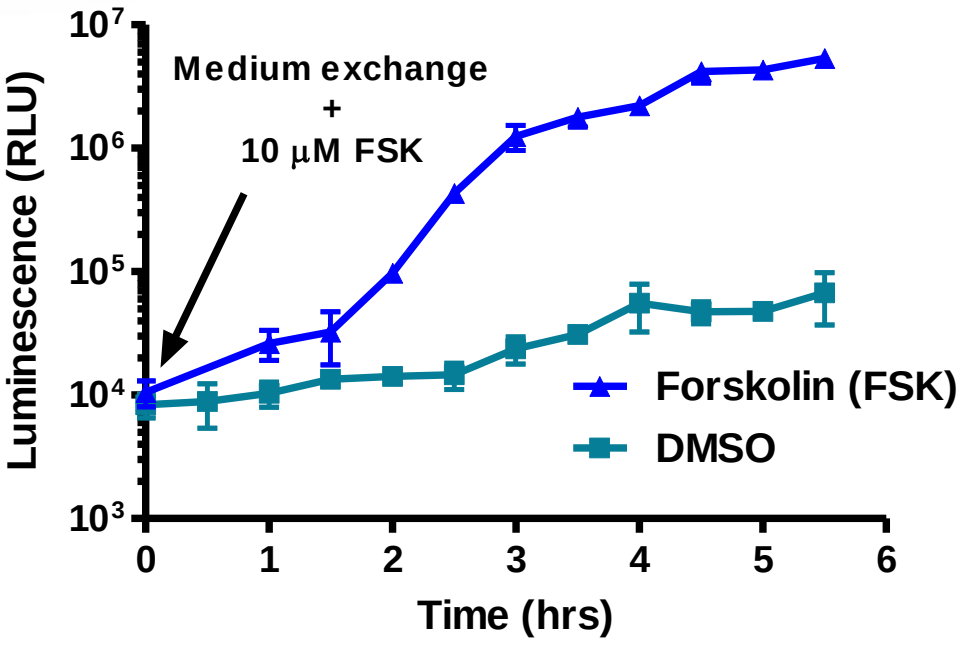
NlucP (636 bp)

Secretion Format

secNluc (597 bp)

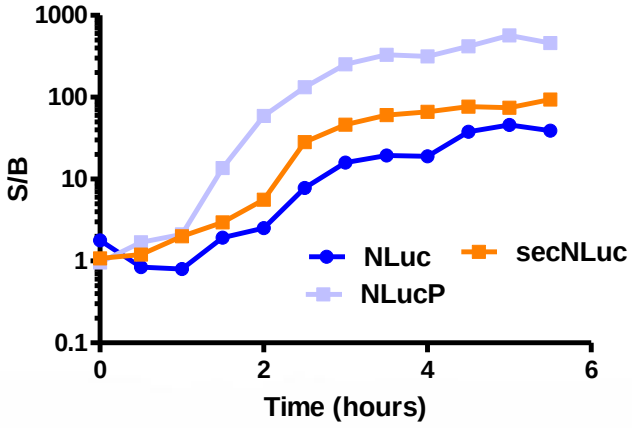
Secretion signal

Secretion based format using secNluc

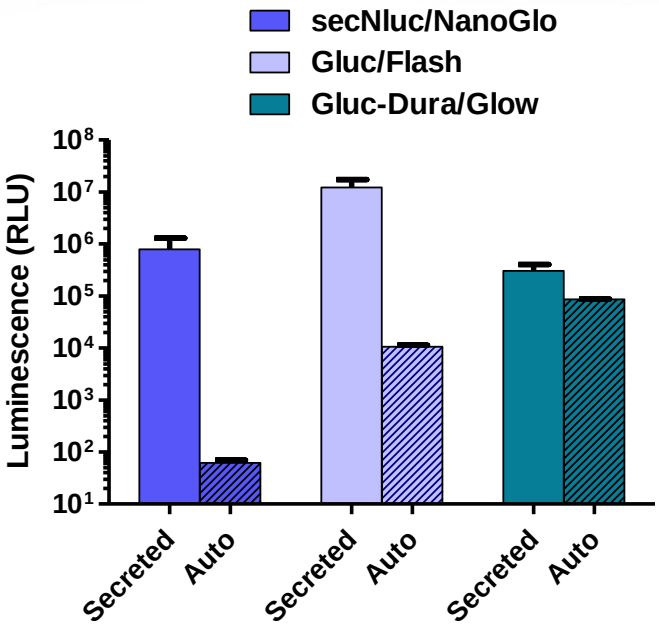


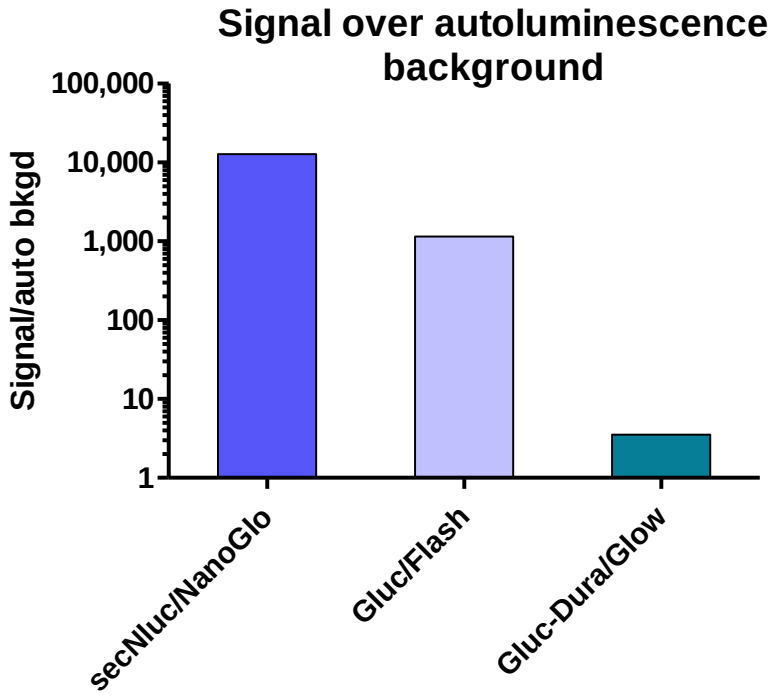
Experimental details: transient transfection of HEK293 cells with CREB inducible construct; addition of 10 µM forskolin at time zero.

- Sample medium at multiple time points without cell lysis
  - Kinetic studies from the same set of wells
- Half-life of secNluc protein > 4 days at 37°C in medium
- Response dynamics similar to unfused Nluc
- Similar pharmacology vs. Nluc/NlucP



Gluc kits: bright, but high autoluminescence background





Gluc kits: high background limits sensitivity & dynamic range

Experimental details: HepG2 cells (DMEM +10% FBS) transiently transfected w/CMV promoter constructs; removal of aliquots after 22 hrs; n = 12 per treatment.

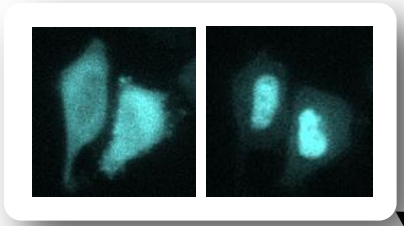
NanoLuc™ Luciferase as a protein function probe

Applications of full-length NanoLuc™ Luciferase.

Promega

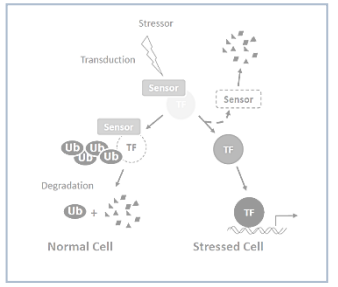
NanoLuc™ Luciferase as a fusion partner: Proof of concept experiments

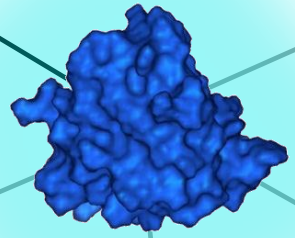
Promega

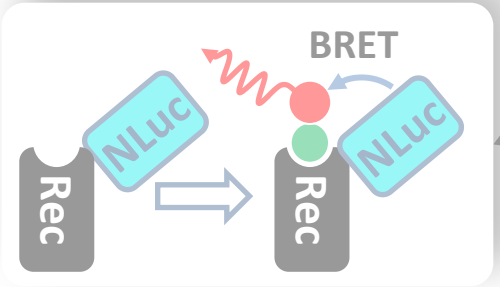


Protein Translocation

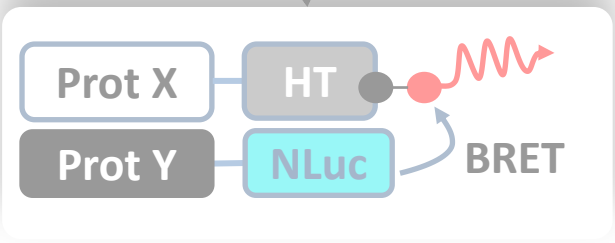
Protein Stability



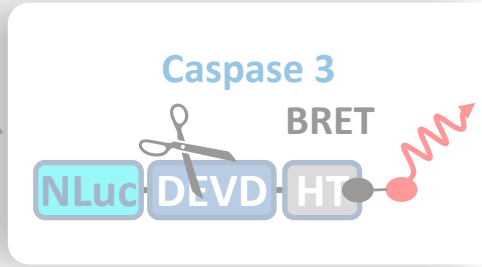




Receptor Interactions



Protein-Protein Interactions



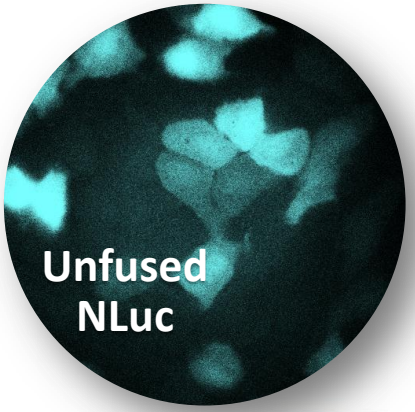
Biosensors

NanoLuc™ Luciferase excels in bioluminescent imaging applications

Promega

Nluc brightness leads to short exposure times:

- Fluc/Rluc: 1-5min/exposure
- Nluc: 1-5sec/exposure



NanoLuc & LV200 featured @ASCB 2012 Olympus Product Showcase

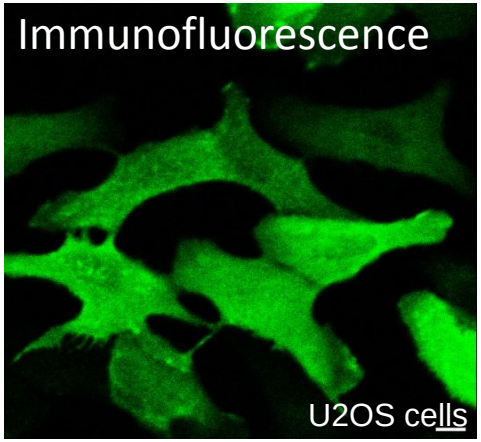
Why bother? Fluorescence works.

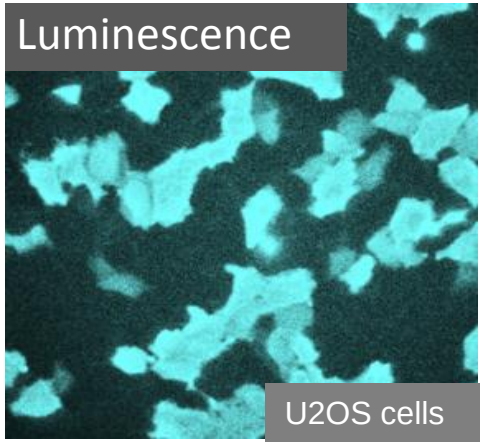
- Fluors are susceptible to photobleaching.
- Excitation can cause autofluorescence of other fluors
- Luciferases will generate light as long as substrate is available

Olympus LV200
Bioluminescence Imager

NanoLuc is well behaved in mammalian cells

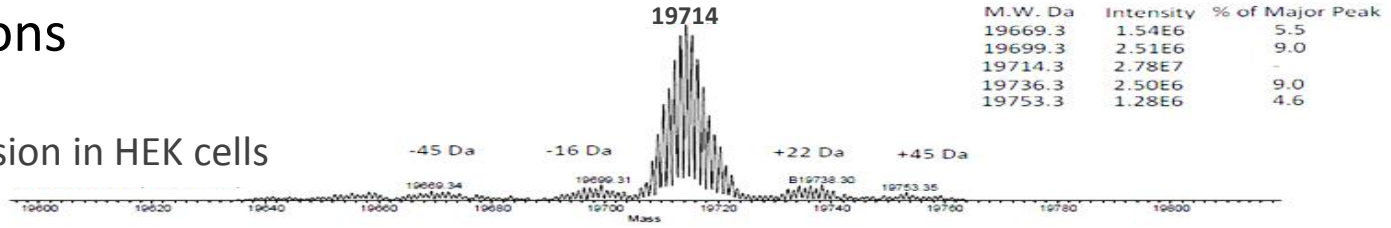
- Uniform intracellular distribution



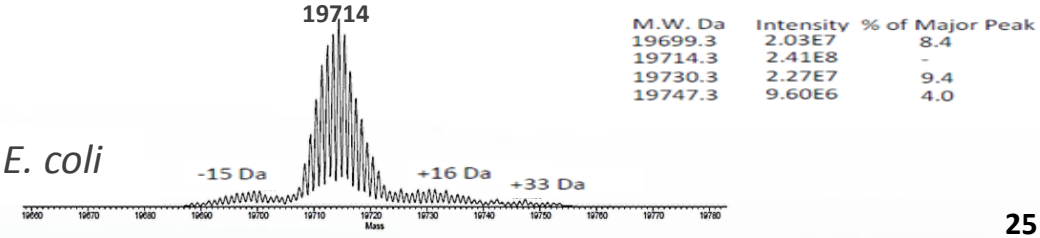


- No post-translational modifications

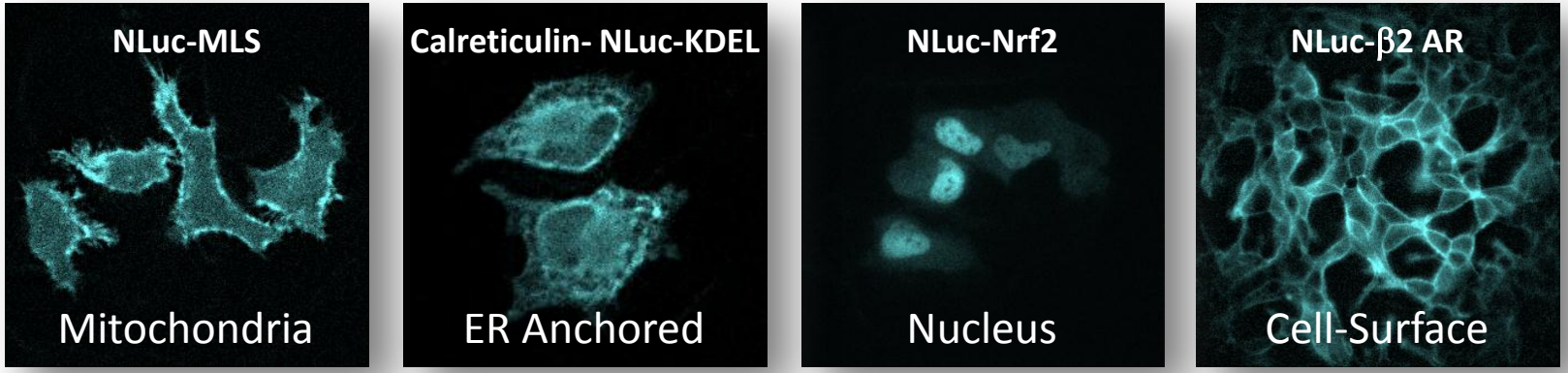
Expression in HEK cells



Expression in *E. coli*



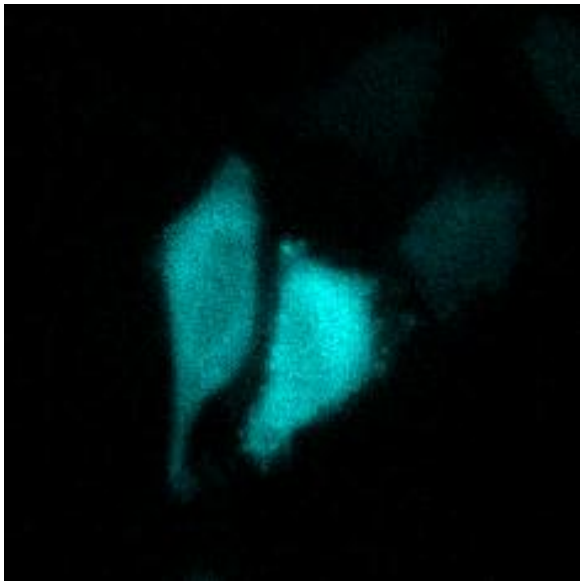
NanoLuc™ Fusions can be designed to go anywhere



NanoLuc™ Luciferase fusions could be a useful tool to investigate cell biology

Bioluminescence imaging of protein translocation

cytoplasm → nucleus



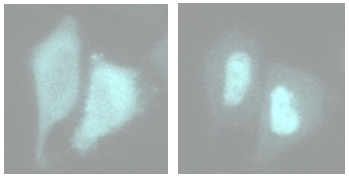
Time lapse: 13 minutes

NanoLuc fusion to
Glucocorticoid Receptor

HeLa cells; 500nM dexamethasone treatment

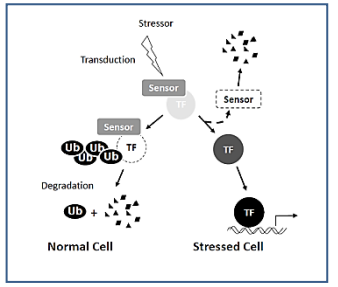
NanoLuc™ Luciferase as a fusion partner: Proof of concept experiments

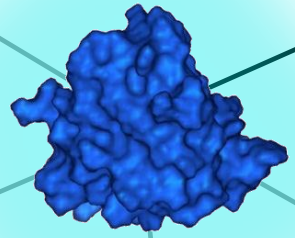
Promega

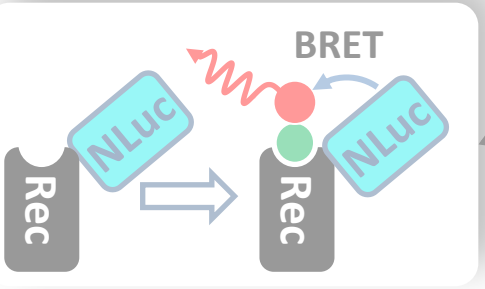


Protein Translocation

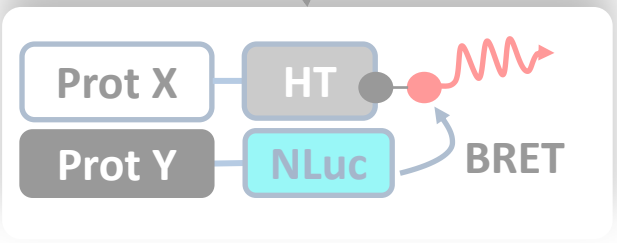
Protein Stability



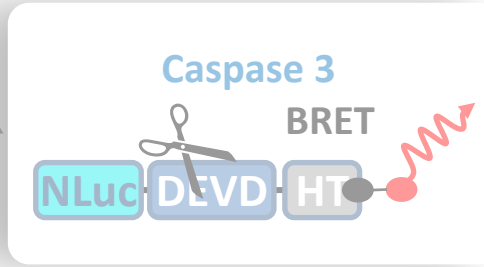




Receptor Interactions



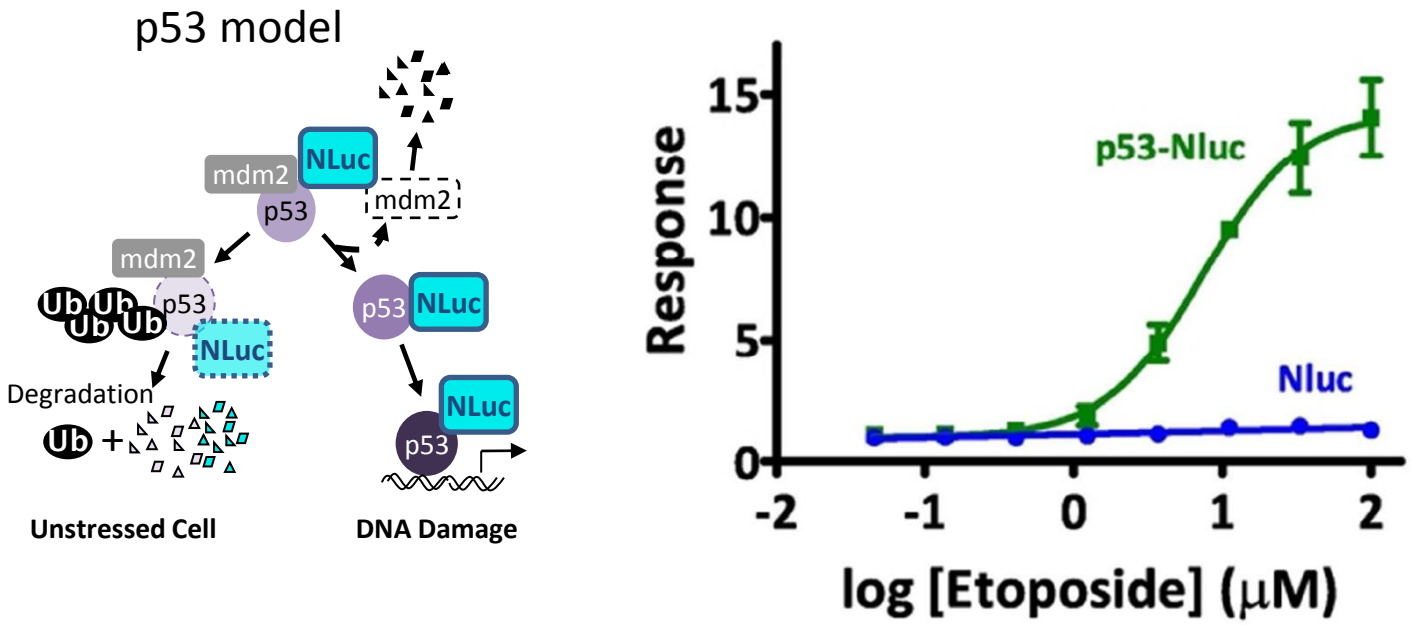
Protein-Protein Interactions



Biosensors

Monitoring Protein Stability with NanoLuc™ Luciferase

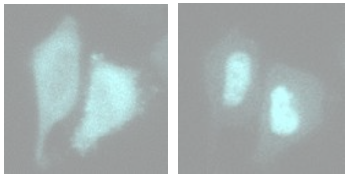
NanoLuc™ Luciferase can be added to a protein as a probe for protein stability.



The fusion can be used as a probe of stability

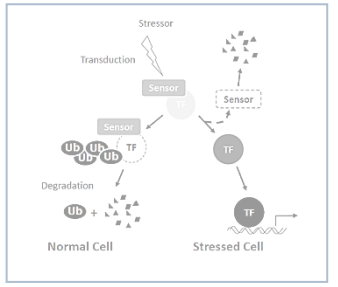
NanoLuc™ Luciferase as a fusion partner: Proof of concept experiments

Promega

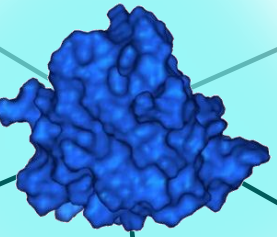


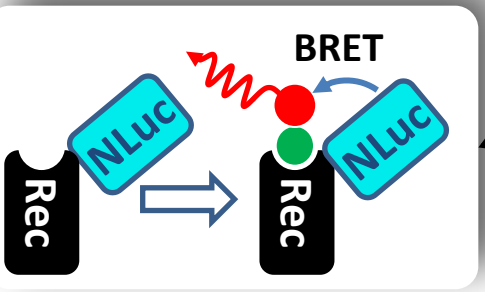
Protein Translocation

Protein Stability



NLuc

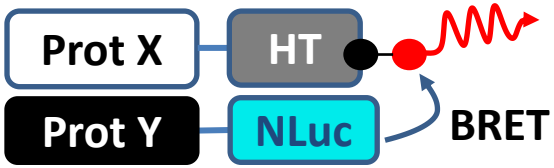




Receptor Interactions

Caspase 3

Biosensors

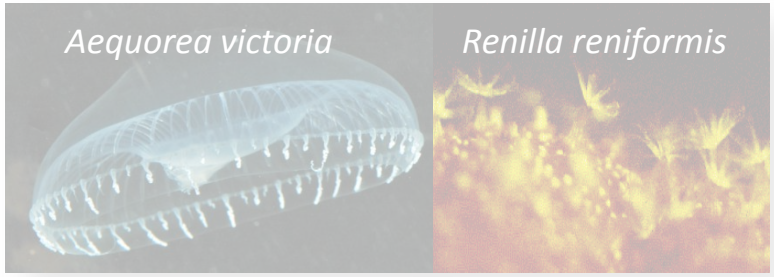


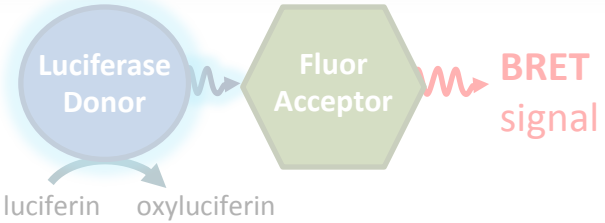
Protein-Protein Interactions

Bioluminescence Resonance Energy Transfer (BRET)

Promega

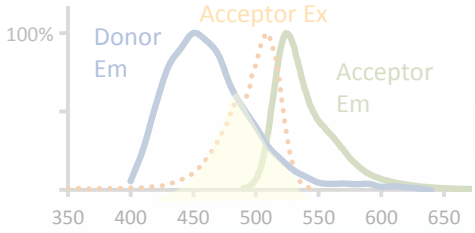
...in Nature



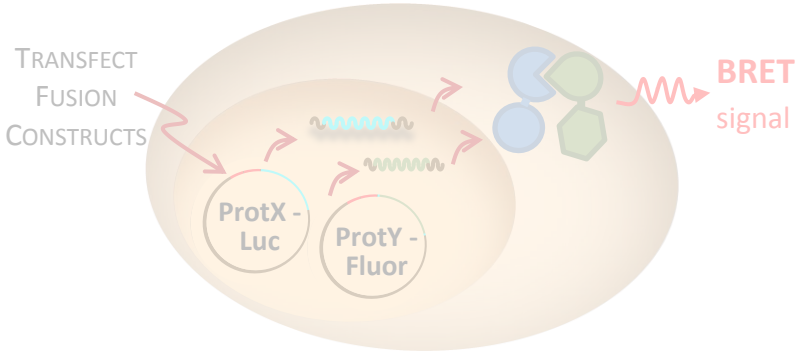


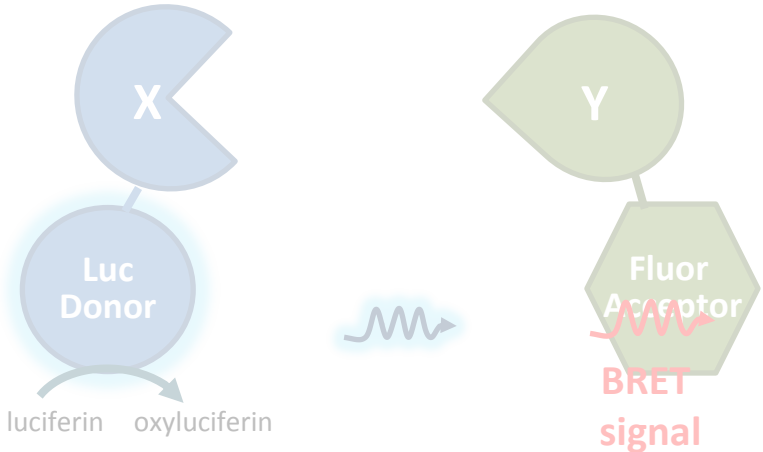
Requirements for BRET:

- Donor & Acceptor must be close (10-100 Å)
- Donor emission spectrum must overlap with Acceptor excitation spectrum



...in the Lab





first described by C.H. Johnson & colleagues in 1999

Could NanoLuc™ work better as a BRET donor?

BRET-beneficial properties of NanoLuc Luciferase:

100x brighter than Rluc…

- ✓ less spectral overlap of Donor & Acceptor needed
- ✓ Better S:B and dynamic range

Blue-shifted emission…

- ✓ Lots of space on the spectrum for the Fluor Acceptor

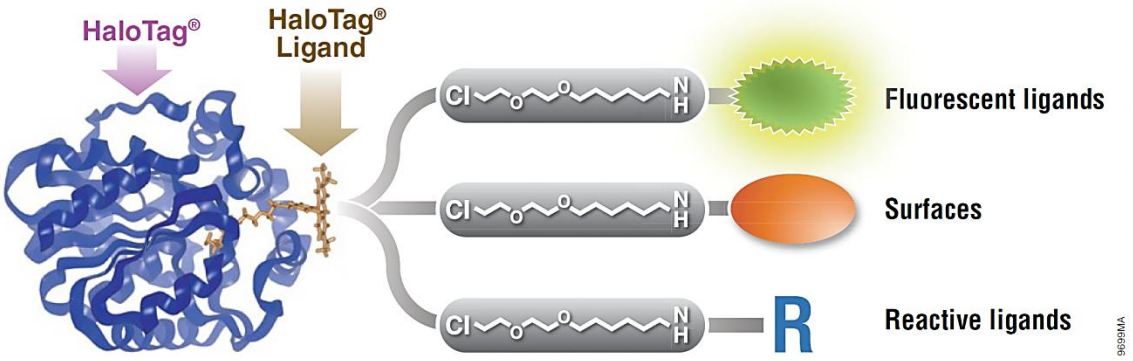
Nluc 460nm; Rluc 480nm; Fluc 560nm

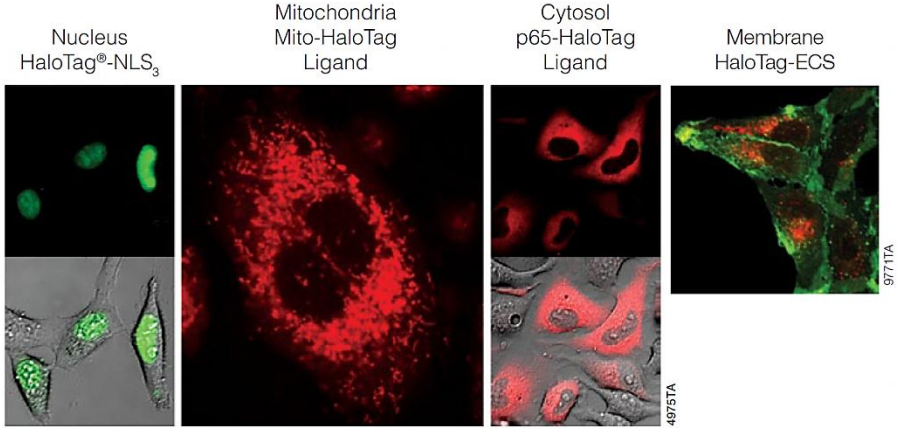
We have a potential acceptor fusion protein:

HaloTag®

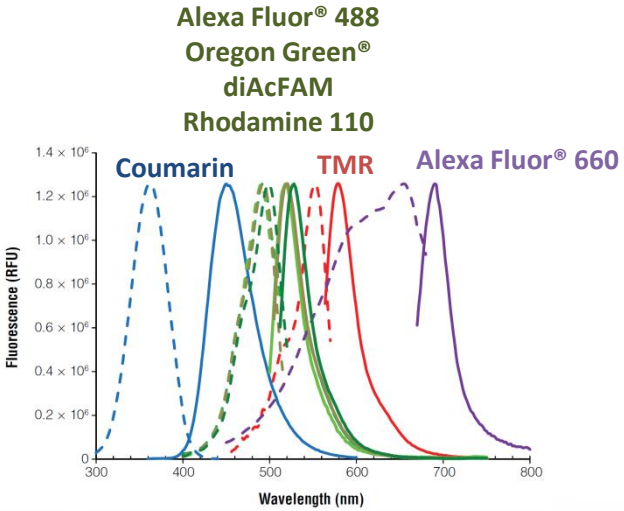
34.1kDa protein engineered from halophilic bacterial hydrolase

- Forms **covalent attachment** to functional ligand
- Add ligand to cells expressing HaloTag fusion





Goes anywhere in the cell

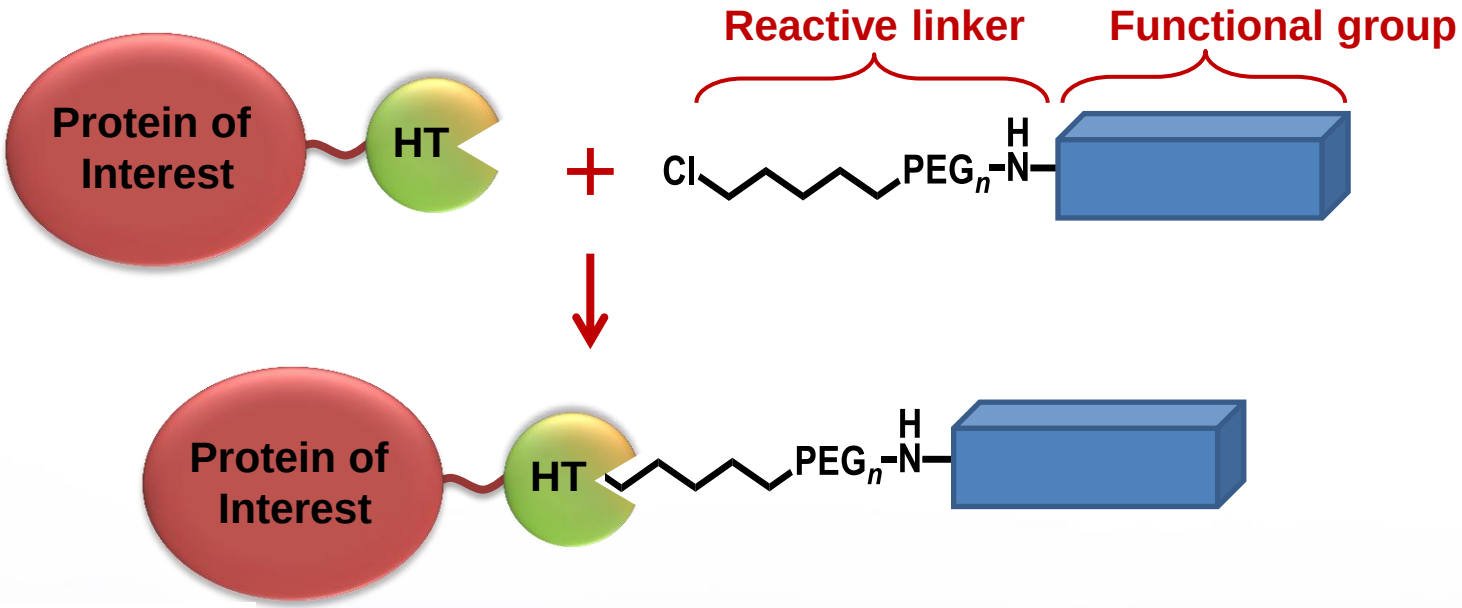


Variety of fluors ready-to-use

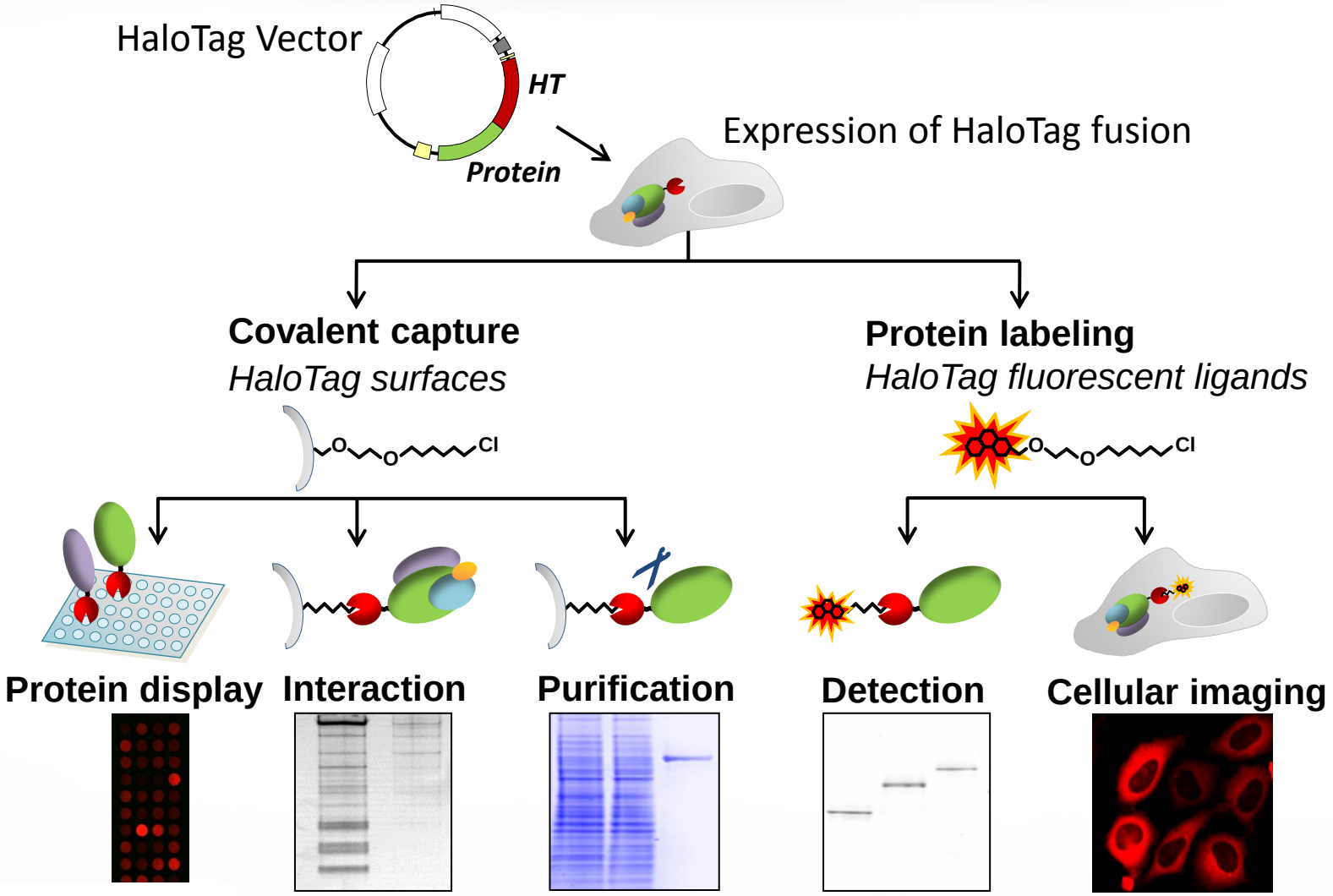
HaloTag technology – affinity tag alternative

Protein-mediated covalent attachment:

- *Strong binding – covalent bond is essentially irreversible*
- *High specificity – modified catalytic mechanism*
- *High binding rate – optimized protein structure*

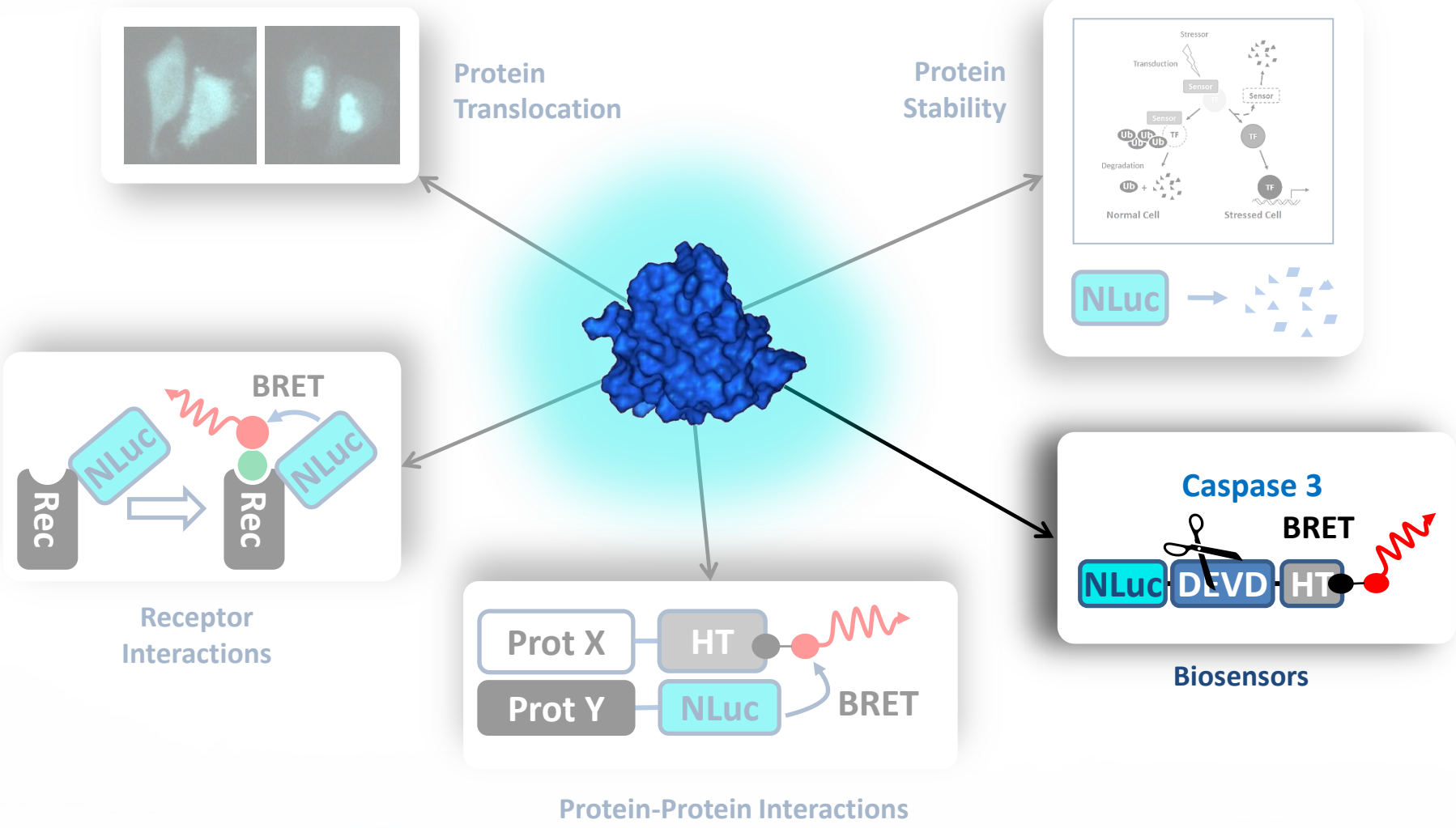


Multiple uses with one genetic construct

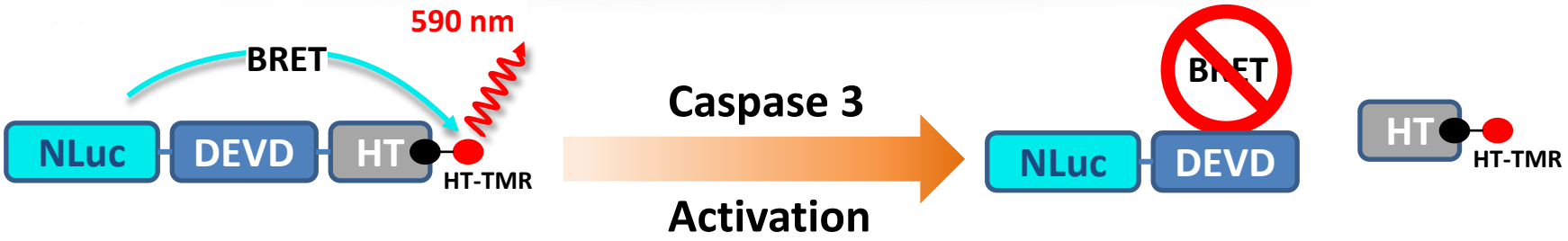


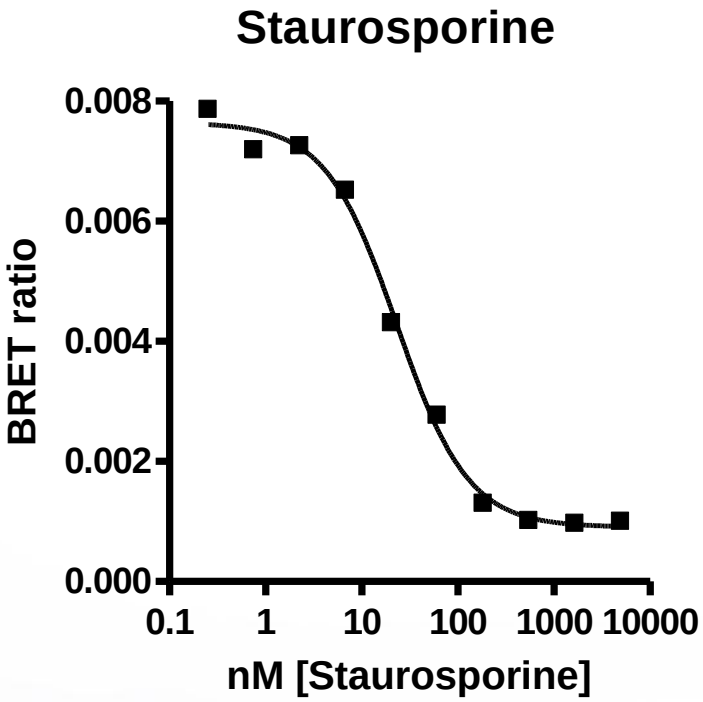
NanoLuc™ Luciferase as a fusion partner: Proof of concept experiments

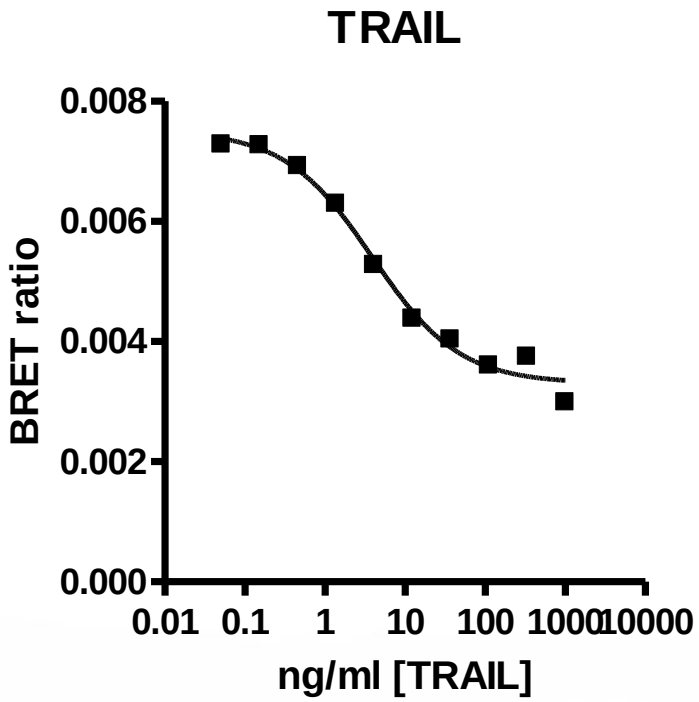
Promega



Biosensor: Disrupting BRET with a cleavable bridging peptide sequence

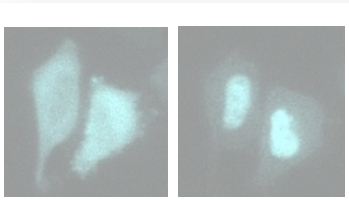






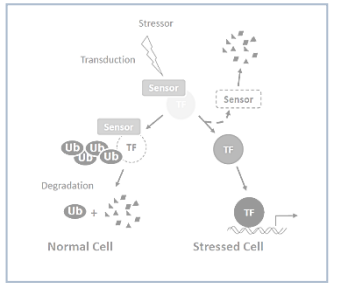
NanoLuc™ Luciferase as a fusion partner: Proof of concept experiments

Promega

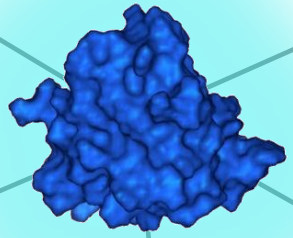


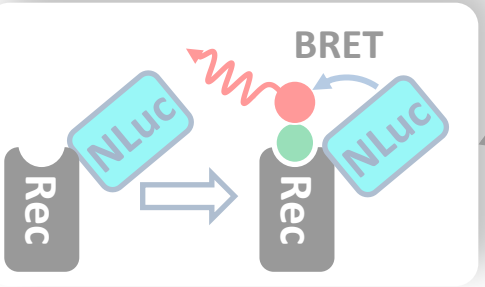
Protein Translocation

Protein Stability



NLuc

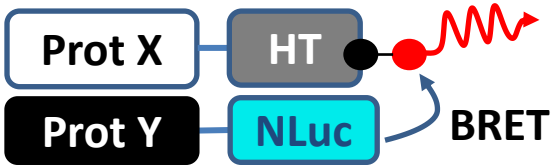




Receptor Interactions

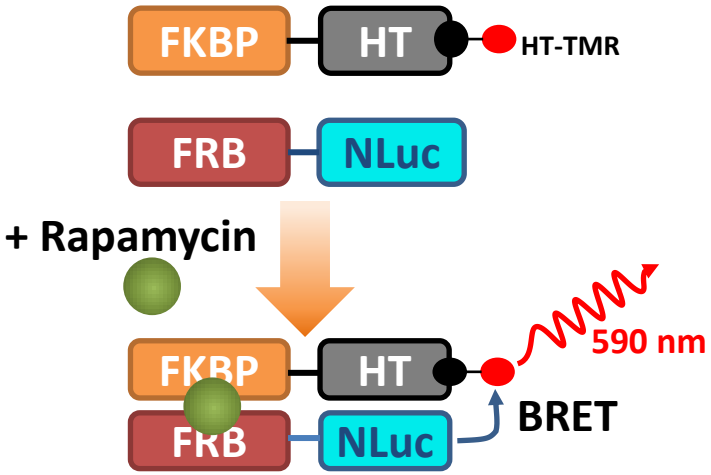
Caspase 3

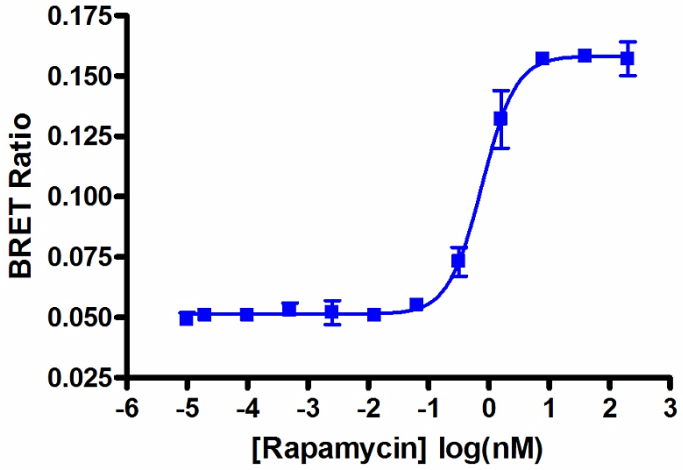
Biosensors



Protein-Protein Interactions

Can NLuc:HT Pair be used for Protein-Protein BRET?

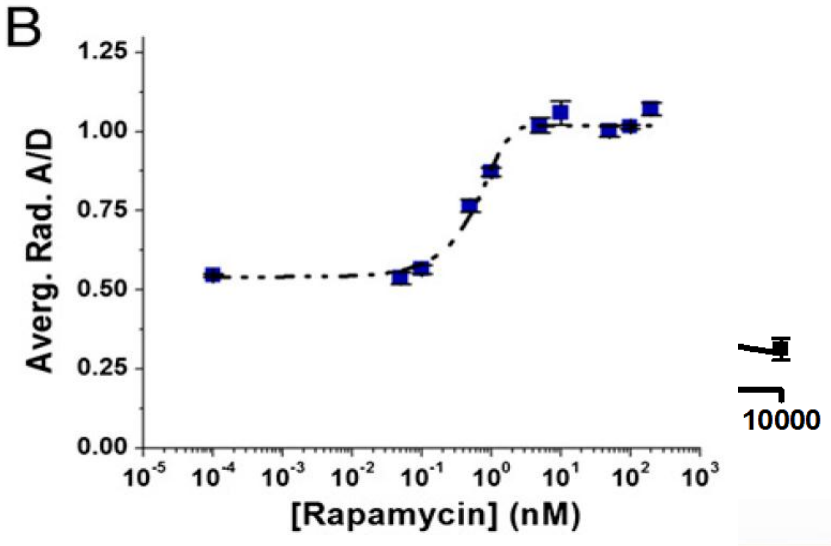




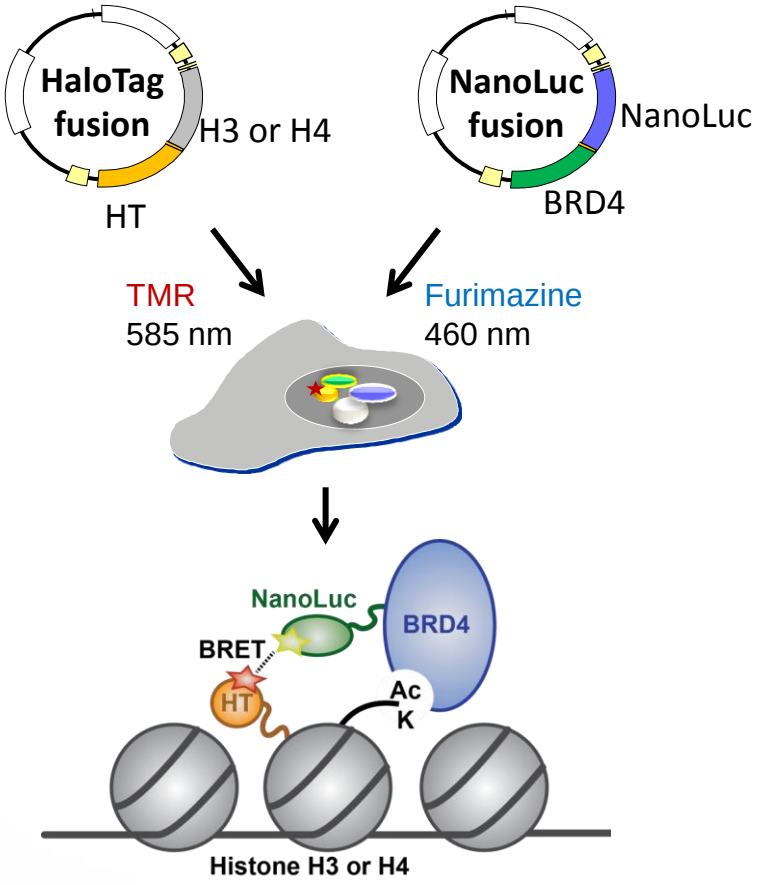
Same model system used with BRET 6 System

RLuc8.6 → **TurboFP**

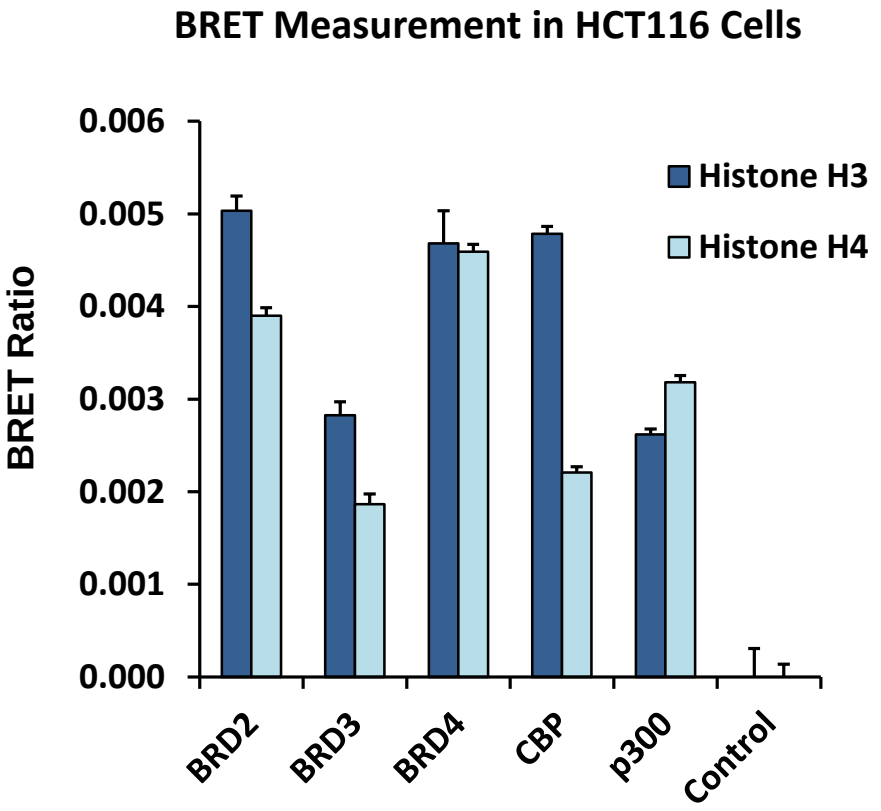
Dragulescu-Andrasi, A., et al (2011) *PNAS* **108**, 12060-5.



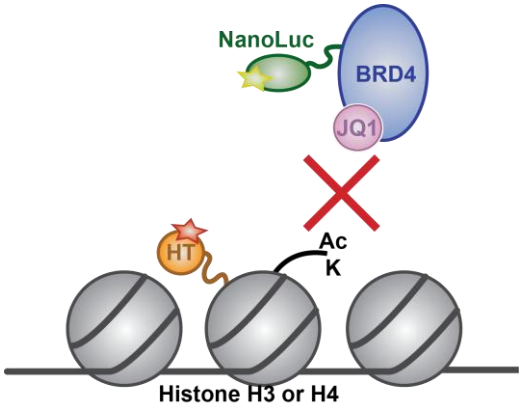
BRET assay for detecting bromodomain interactions with histones



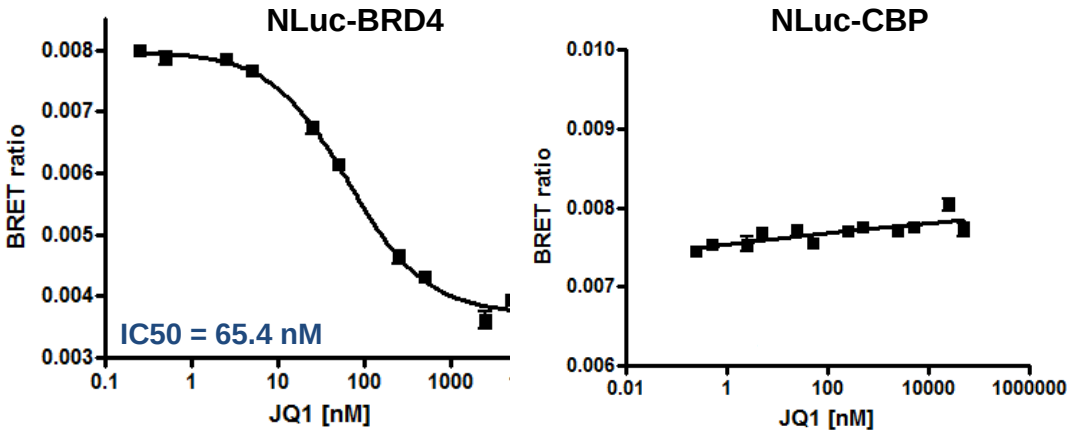
BRET (600/480nm) Measurement



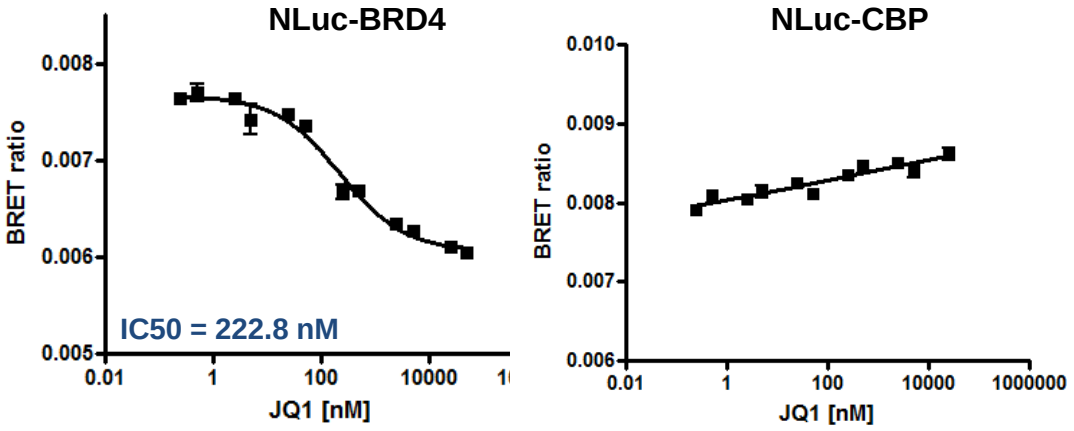
Measuring effect of BET inhibitor on histone-bromodomain interactions



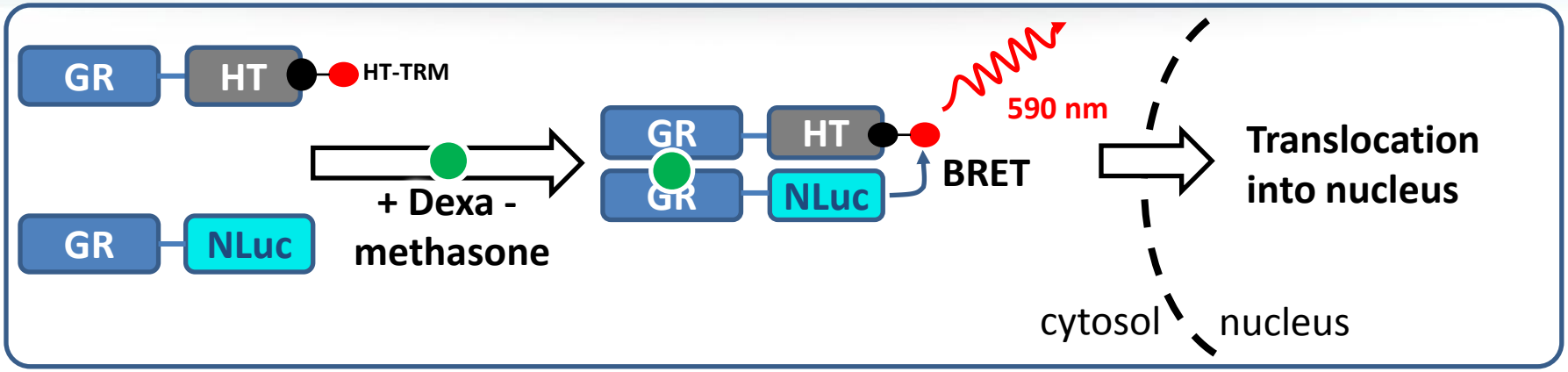
Histone H3.3-HaloTag

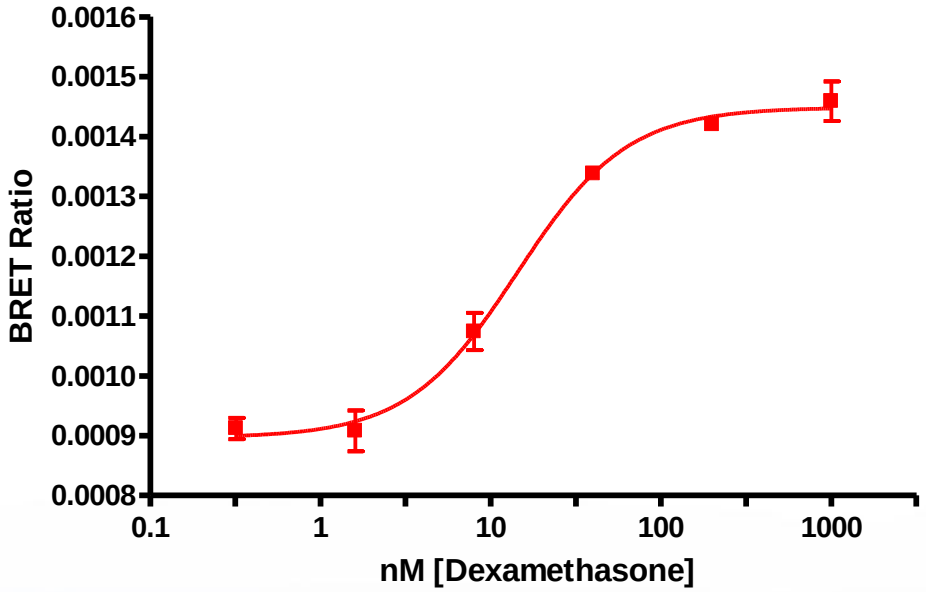


Histone H4-HaloTag

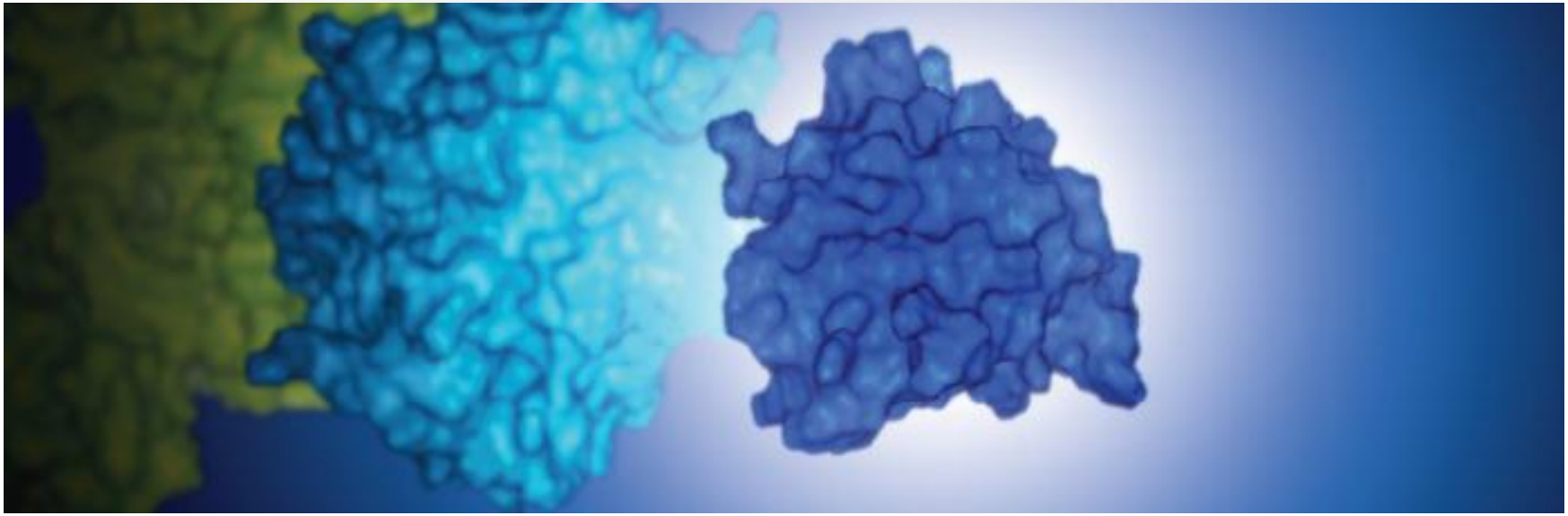


Glucorticoid Receptor Dimerization





Bright Future for NanoLuc™ Luciferase fusions



Brightness improves bioluminescent imaging

Versatility to go anywhere in cell

Versatility to allow stability measurements

Brightness allows BRET with HaloTag® Fusions

- Biosensors
- Protein:Protein Interactions

Brightness allows BRET with fluorescent ligands

- Ligand binding assays

Nano-Glo™ Luciferase Assay System

Just make enough 1X Nano-Glo Assay Reagent from the 50X substrate and buffer to meet your needs. No need to make up all the reagent at once.

Cat # N1110: 10ml (100 assays)

- 200µl Nano-Glo™ Luciferase Assay Substrate
- 10ml Nano-Glo™ Luciferase Assay Buffer

Cat # N1130 10x10ml (1,000 assays)

- 10 x 200µl Nano-Glo™ Luciferase Assay Substrate
- 10 x 10ml Nano-Glo™ Luciferase Assay Buffer

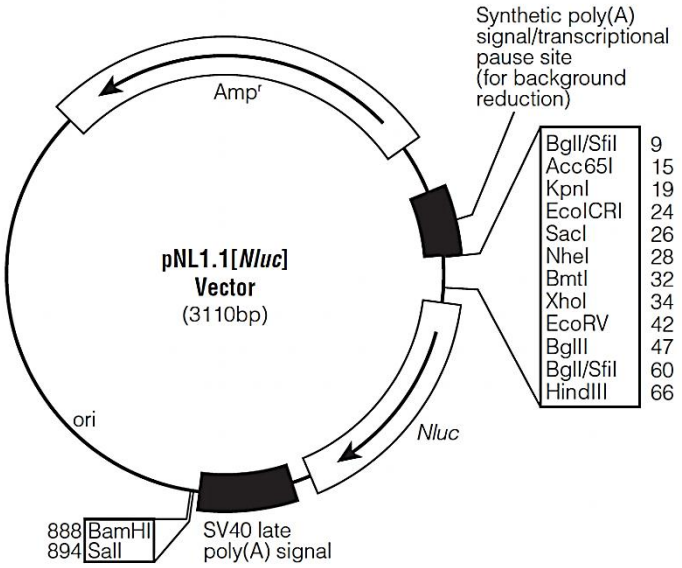
Cat.# N1120 100ml (1,000 assays)

- 2 x 1ml Nano-Glo™ Luciferase Assay Substrate
- 100ml Nano-Glo™ Luciferase Assay Buffer

Cat.# N1150 10x100ml (10,000 assays)

- 5 x 4ml Nano-Glo™ Luciferase Assay Substrate
- 10 x 100ml Nano-Glo™ Luciferase Assay Buffer

Currently Available Vectors



The pNL vectors are built on the pGL4 Vector backbone allowing transfer of inserts from pGL4 series vectors to the pNL vectors.

Plasmid	Reporter	Marker	Promoter	MCS	Catalog #
pNL1.1	Nluc	-	-	Y	N1001
pNL1.2	NlucP	-	-	Y	N1011
pNL1.3	secNluc	-	-	Y	N1021
pNL2.1	Nluc	Hygro	-	Y	N1061
pNL2.2	NlucP	Hygro	-	Y	N1071
pNL2.3	secNluc	Hygro	-	Y	N1081
pNL3.1	Nluc	Hygro	minP	Y	N1031
pNL3.2	NlucP	-	minP	Y	N1041
pNL3.3	secNluc	-	minP	Y	N1051
pNL1.1.CMV	Nluc	-	CMV	N	N1091
pNL1.3.CMV	secNluc	-	CMV	N	N1101
pNL3.2. NF-κB-RE	NlucP	Hygro	NF-κB-RE/minP	N	N1111

Want to know what vectors are coming?

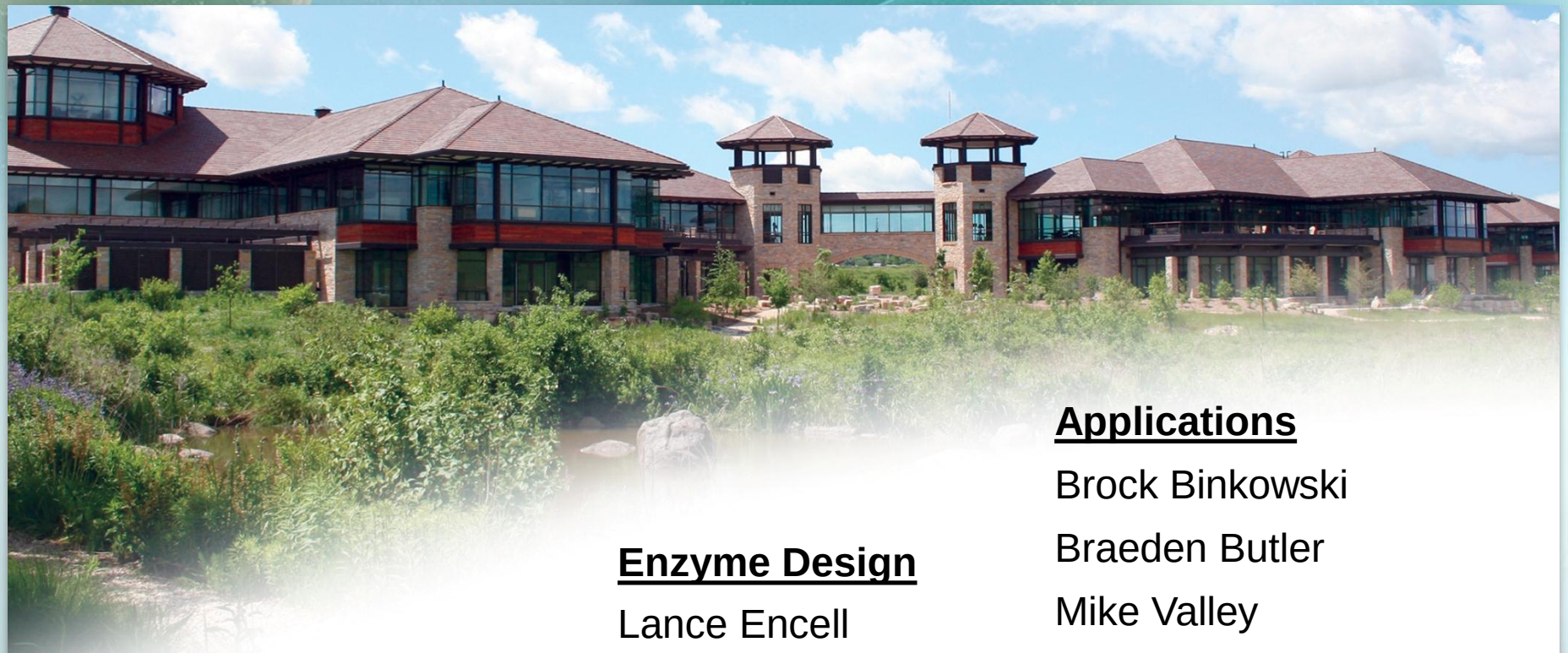
New vectors using Promega exclusive technologies are typically offered as a custom product before they become a catalog product.

Visit the Current Research Materials page at:

www.promega.com/cam

Stay on the cutting edge and get research tools before they are available to everyone!

You can also request construction of a special vector to meet your exact needs with ***Custom Assay Services***



Keith Wood

Substrate Design

Poncho Meisenheimer

James Unch

Ruslan Arbit

Hui Wang

Dieter Klaubert

Enzyme Design

Lance Encell

Monika Wood

Mary Hall

Kris Zimmerman

Paul Otto

Hélène Benink

Gedi Vidugiris

Mike Slater

Applications

Brock Binkowski

Braeden Butler

Mike Valley

Matt Robers

Thomas Machleidt

Chris Eggers

Frank Fan

Danette Daniels

Marie Schwinn

Jacqui Mendez

Questions Welcome