horizon

precision genome editing

Translating Genomes | Personalizing Medicine

X-MAN™ reporter cell lines: Tools to study endogenous promoter activity and protein dynamics

March 2014

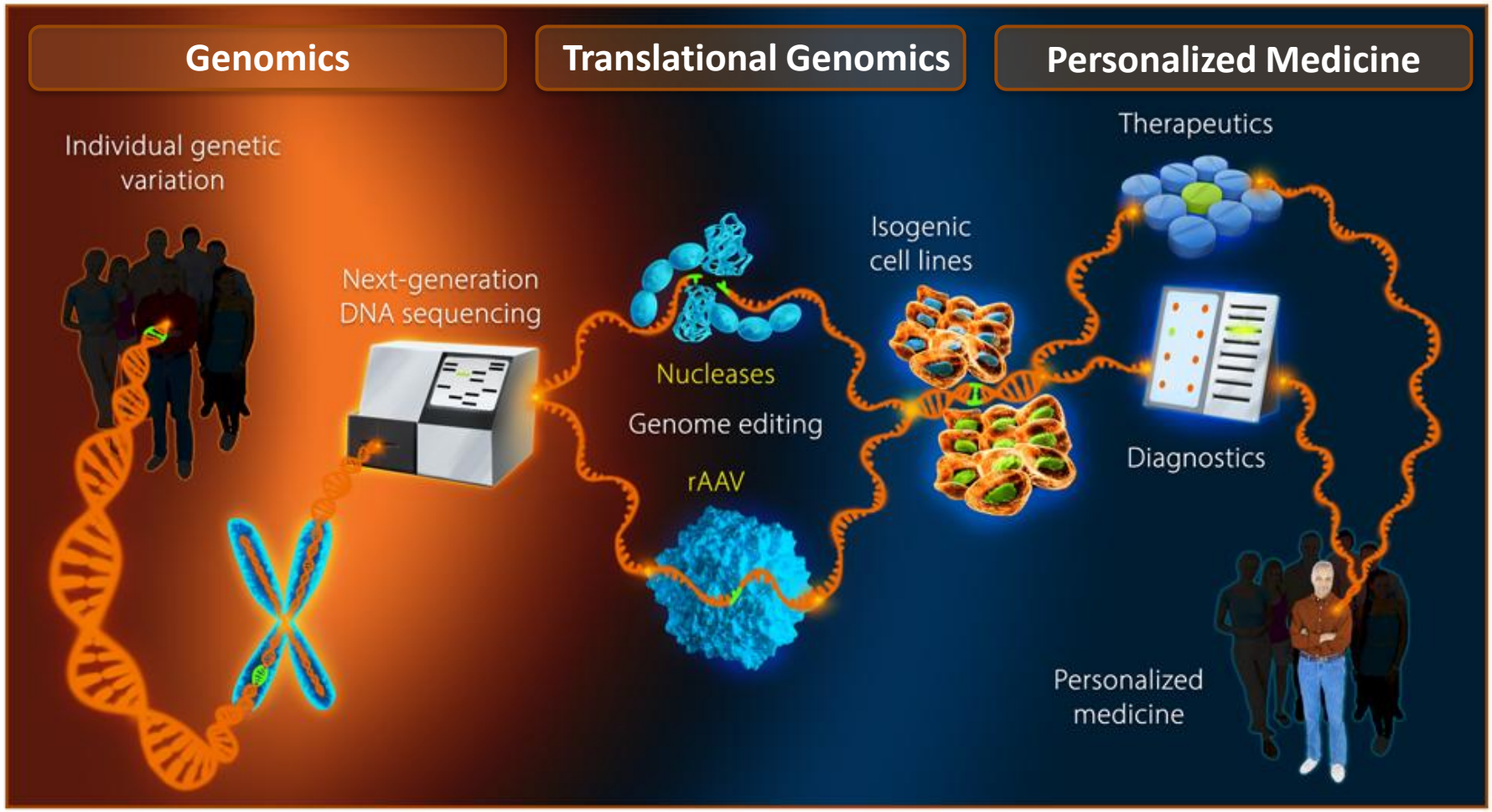
SCRIP AWARDS 2013 WINNER

THE QUEEN'S AWARDS FOR ENTERPRISE: 2012

Agenda

- Introduction to Horizon's Technology
- X-MAN™ HaloTag® Reporter Kits
- X-MAN™ NanoLuc™ Reporter Kits

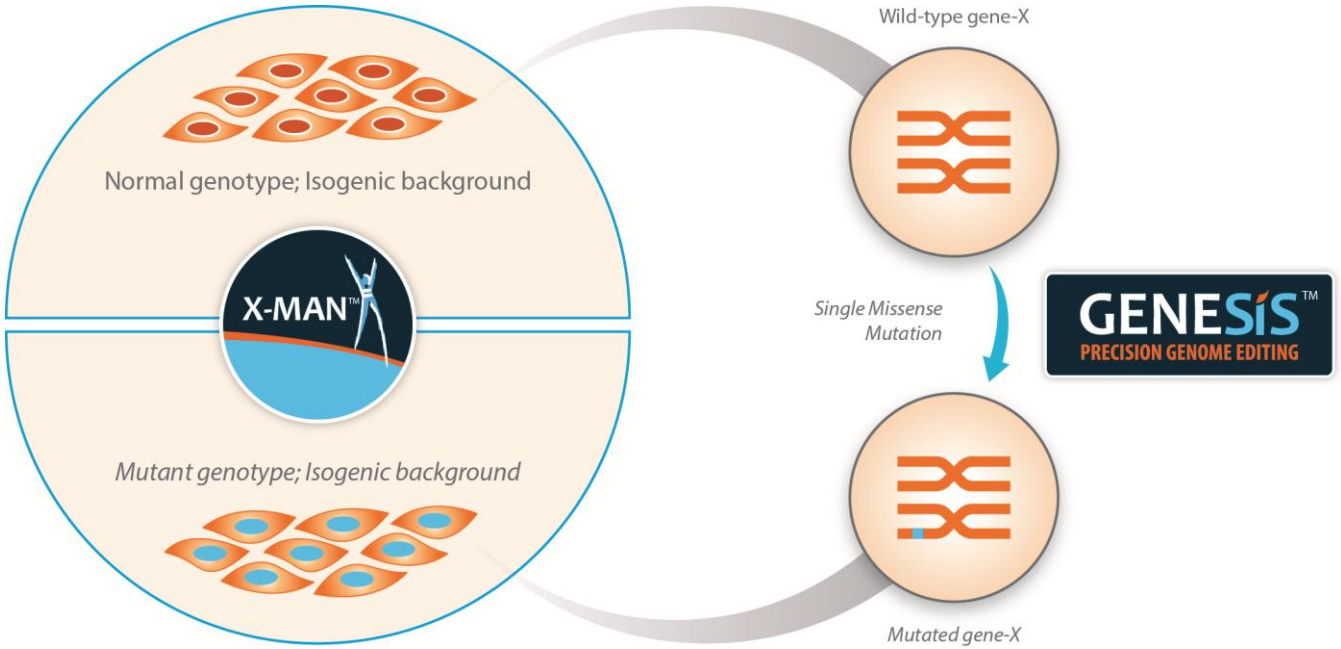
Entering the post-genomic era: Translating genetic information into personalised medicine



- Genome sequencing is now routine and emphasis is shifting to the 'functional human genome'
- Horizon are experts in 'Genome Editing' in human/mammalian cells – **targeted gene KI/KOs**
- **An essential new tool to model patient genetics *in vitro* and discover novel targets, Tx's and Dx's**

GENESIS™: Horizon's gene editing platform & X-MAN™ cell lines

- GENESIS™ platform consists of **rAAV and nuclease-based** gene editing technologies
- Generated over 500 genetically-defined X-MAN™ isogenic human cell lines
  - **KOs** to study gene function or target validation
  - Accurate **KI-models** of target patient genetics **"surrogate patients" + perfect normal control**
- **Endogenous pathway reporter** lines



- **GENESIS™** Genome Editing: Make any mutation of interest
- Validate putative disease-causing mutations and SNPs
- Definitively study target biology and drug responses
- Create pathway reporter lines
- Build complex diseases from ground up, or sequentially correct them

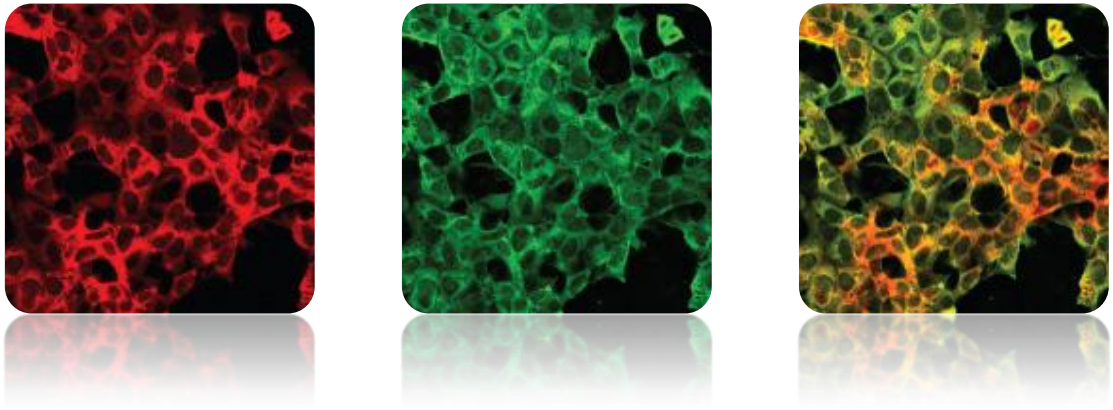
X-MAN™ Reporter Kits: Live cell endogenous pathway reporters

- rAAV genome editing used to engineer endogenous tags and reporters
- Available in kit format from Horizon
  - **X-MAN™ NanoLuc™ Reporter Kits**
    - Knock-in of Luciferase reporter into endogenous locus for *in vitro & in vivo pathway* read-outs
  - **X-MAN™ HaloTag® Reporter Kits**
    - Knock-in of reporters/tag into endogenous locus for *in vitro* imaging / pull-downs / purification

Kits contain cell lines and all reagents necessary to perform a set of experiments

horizon precision genome editing

Promega

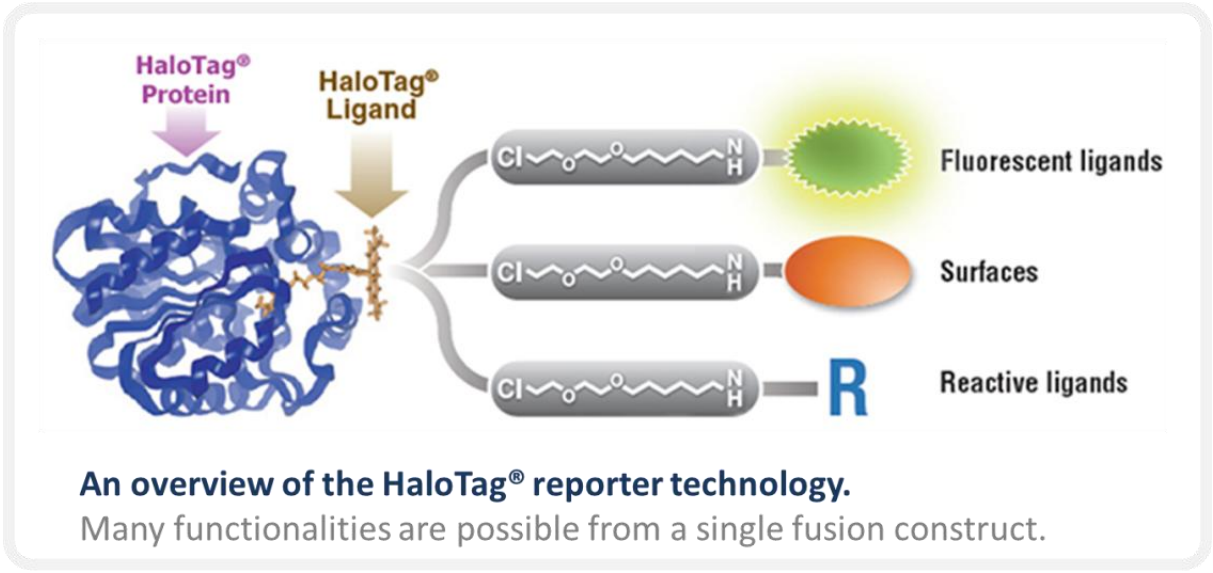


Agenda

- Introduction to Horizon's Technology
- X-MAN™ HaloTag® Reporter Kits
- X-MAN™ NanoLuc™ Reporter Kits

X-MAN™ HaloTag® Reporter Lines: Technology Background

- X-MAN™ HaloTag® reporter cell lines have been generated using Horizon Discovery's GENESIS™ gene editing platform
- This technology enables introduction of HaloTag® as N- or C-terminal fusions to genes encoding proteins of interest at their native genetic loci, occurring at physiologically relevant levels
- HaloTag® reporter technology has many applications
  - No limit to the experiments that can be performed, only by the genes that you would target
  - Endogenous high-content screening



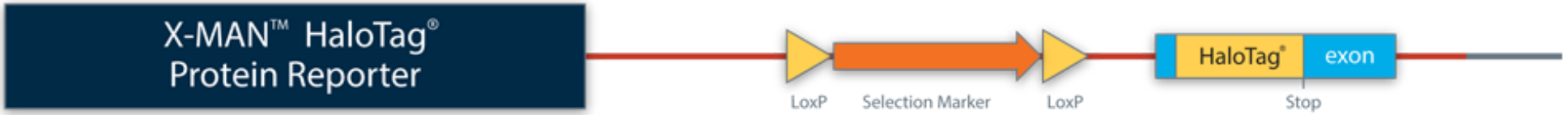
An overview of the HaloTag® reporter technology.
Many functionalities are possible from a single fusion construct.

Flexible and efficient system …. Wider experimental range … Reliable results

X-MAN™ HaloTag® Reporter Kit

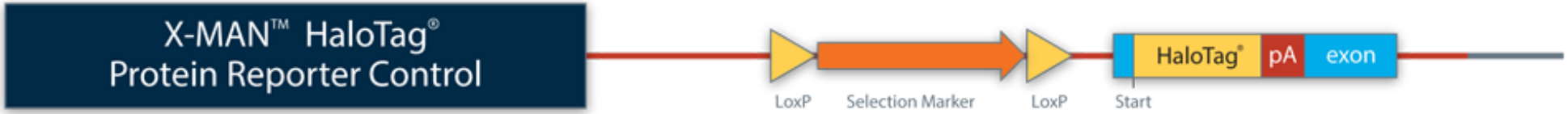
X-MAN™ HaloTag® Protein Reporter Kit

- Engineered to generate N- or C-terminal fusion of HaloTag® to the endogenous protein of interest
- Ideal for studying a wide variety of properties of the protein of interest due to the broad range of HaloTag® ligands available



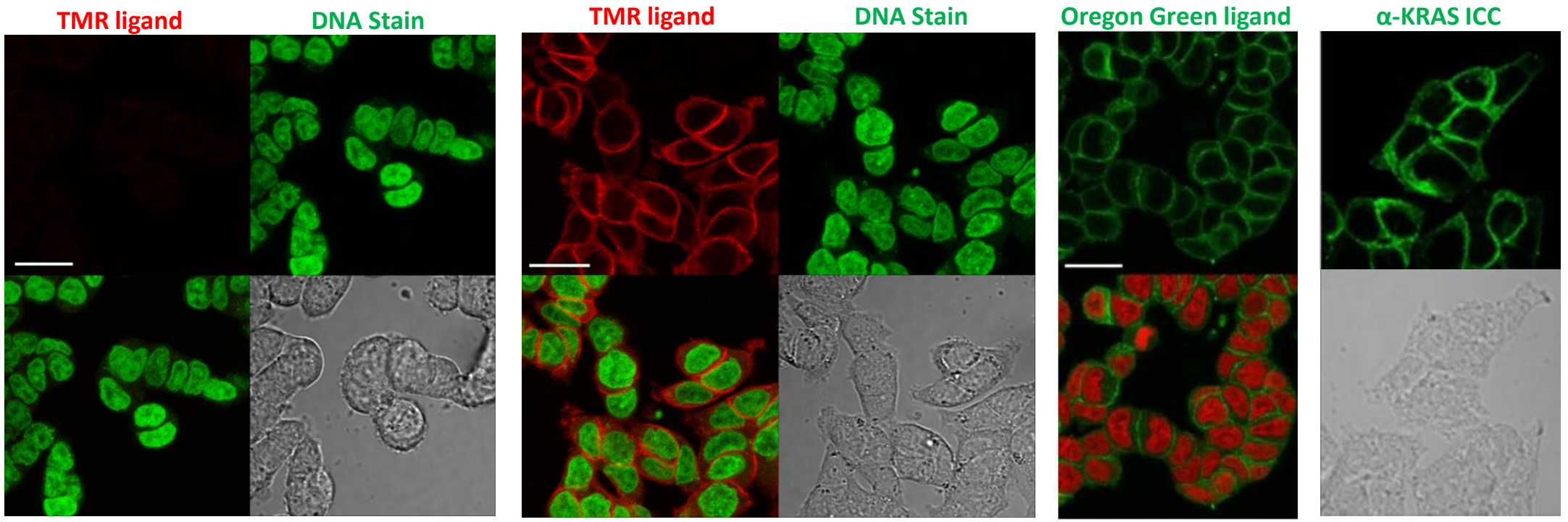
X-MAN™ HaloTag® Protein Reporter Control

- Can be used to demonstrate that the property is specific to the protein of interest, and not conferred by inherent properties of the HaloTag® protein
- Controls for protein reporter lines



HaloTag®: Live cell imaging of protein localisation (K-Ras)

- Some targets/diseases you may want to screen directly for translocation events in live cells
- Easily assayed using HaloTag® and cell-permeable fluorescent HT-ligands, available in different colours
- Use of HaloTag® ligand shows selective staining for HaloTag® engineered lines over unmodified parental cells demonstrating specificity of signal
- HaloTag® ligand shows comparable results to ICC



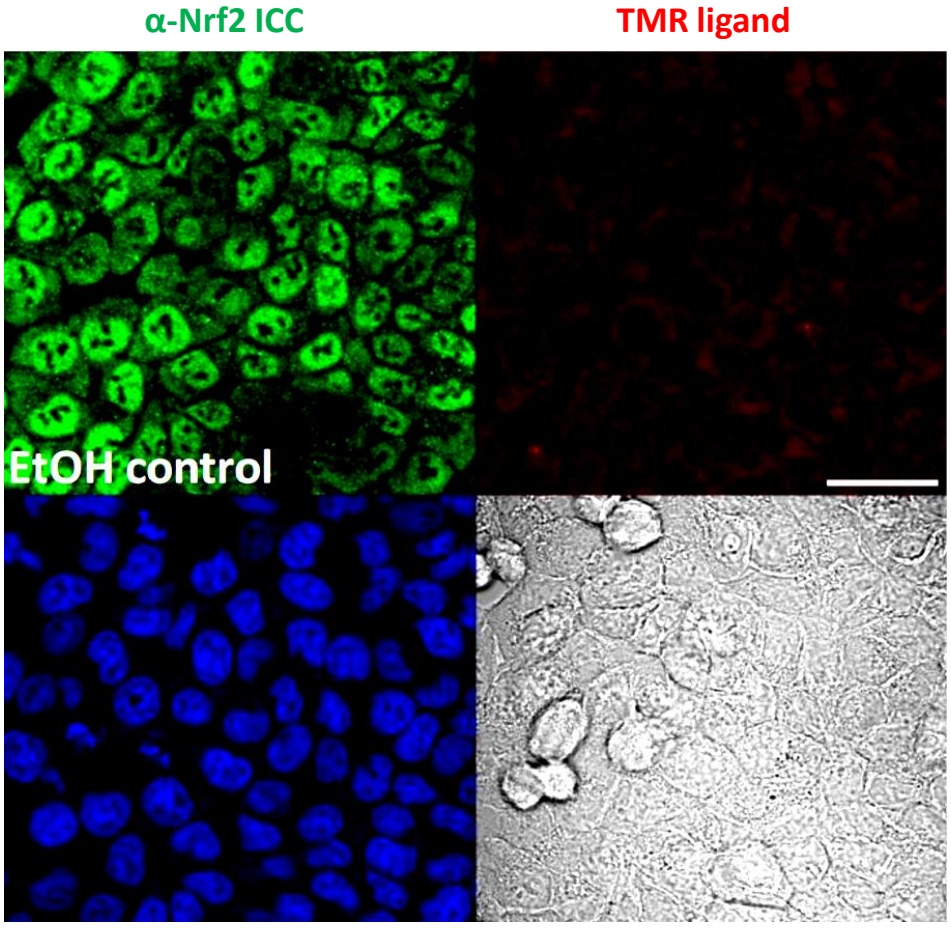
SW48 parental

SW48 X-MAN™ KRAS (+HaloTag®/+) protein reporter

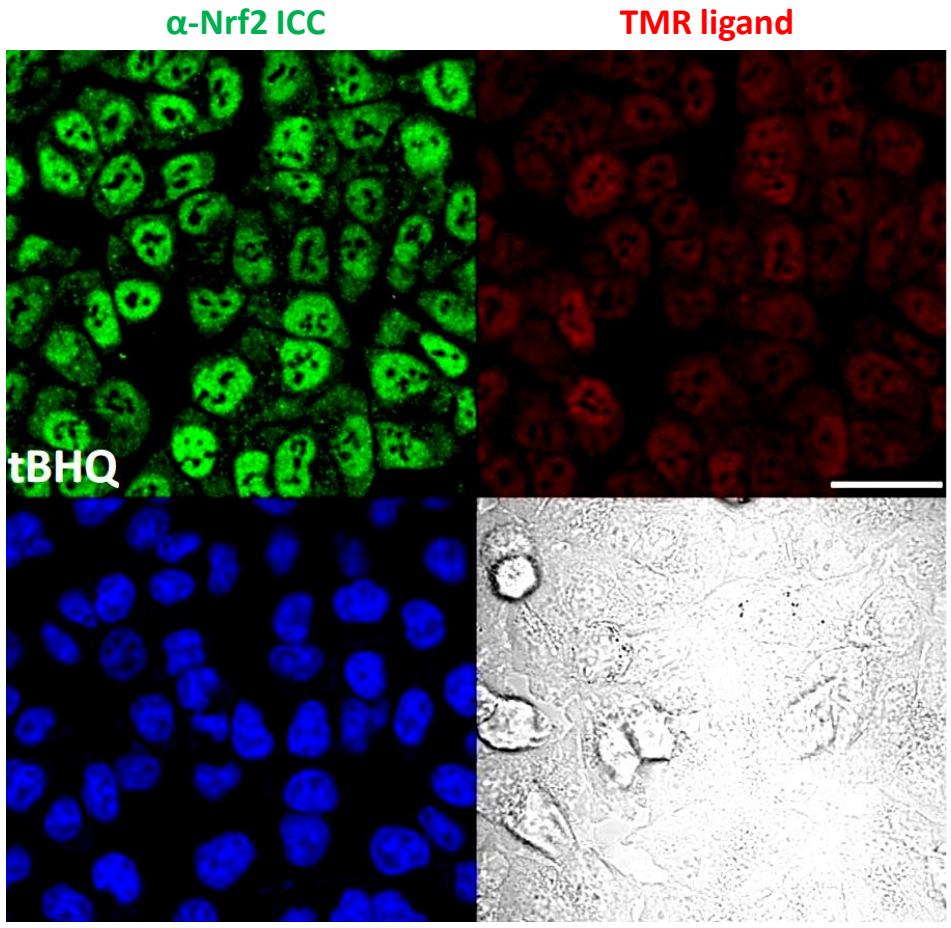
Scale bars = 20 µm

HaloTag®: Can provide superior alternative to ICC

- Well-characterised increase in stability and nuclear translocation of NFE2L2 (NRF2) in response to *tert*-butylhydroquinone (tBHQ) revealed using HaloTag® reporter cell line but not ICC



HCT116 X-MAN™ NFE2L2 (+HaloTag®/+) protein reporter



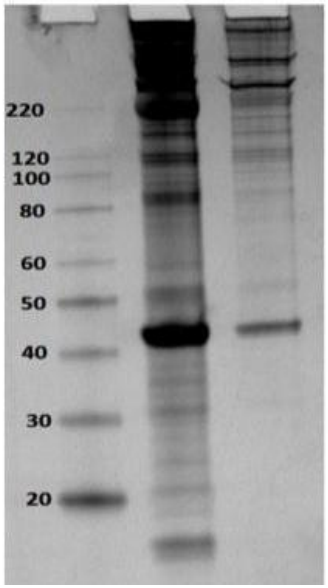
HCT116 X-MAN™ NFE2L2 (+HaloTag®/+) protein reporter

Scale bar = 20 µm

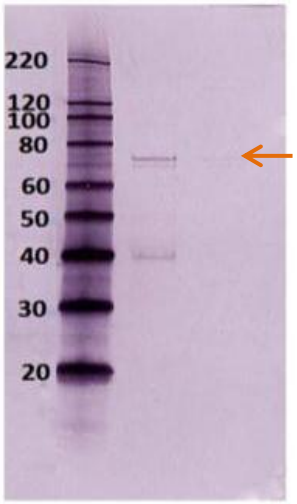
HaloTag®: Pull-down experiments with isogenic reporter lines demonstrate protein enrichment

Protein Enrichment Assays

K-Ras protein (Silver Stain)



Interacting protein (cRAF WB)



Potential Interactors of KRAS
Identified by LC/MS/MS from Halo-KRAS SW48 Horizon Cell line

Signaling Proteins And Exchange Factors	SpC
Rap1B	32
RhoA	25
RAC1	22
RAB8B	20
RhoAAct.	18
FARP1	16
RAB13	14
GNA11	13
RhoG	12
GNA13	10
ATP6V1G1	9
RheB	9
ATP6V1E1	9
HRAS	8
YWHAZ	8
ErbB2	8
RAB7L1	6
ARHGEF12	5

Cytochrome C Subunit II and III	SpC
COX4I1	94
UQCRC1	86
UQCRC2	83
COX6C	46
UQCRFS1	41
MT-CO2	37
COX2	29
CYC1	26
UQCRB	25
COX5A	25
COX5B	23
UQCRQ	13
UQCRH	9
COX6B1	6
COX7A2	4
COX7C	3
UQCR10	3
COX7A2L	3
CYB5B	2
COX11	2

NADH Dehydrogenase	SpC
NDUFS1	77
NDUFA9	41
NDUFS2	38
NDUFA13	34
NDUFV1	33
NDUFB10	28
NDUFA7	26
NDUFA6	26
NDUFA12	24
NDUFS8	21
NDUFS3	19
NDUFB5	17
NDUFA5	17
NDUFB9	16
NDUFS7	15
NDUFV2	14
NDUFB6	12
NDUFS5	11
NDUFA2	10
NDUFB4	10
NDUFAF3	10
NDUFA8	9
NDUFS6	8
NDUFC2	8
NDUFB7	7
NDUFAF4	6
NDUFA10	6
NDUFA3	5
NDUFS4	5
NDUFA4	4
NDUFB3	3
NDUFA11	2

- HaloTag® ligand linked to resin enables stringent pull-downs
- High sensitivity via covalent interaction with matrix

X-MAN™ HaloTag® Kits Currently Available

NCBI Gene Name	Common name	X-MAN™ (+HaloTag®/+) protein reporter	X-MAN™ (+HaloTag®/+) protein reporter of mutated variants	X-MAN™ HaloTag® protein reporter control
HIF1A	HIF1α	x		x
NFE2L2	NRF2	x		x
KRAS	K-Ras	x	G12C	x
			G12D	
			G12V	
			G13D	

- Additional lines are constantly being developed by Horizon or can be developed as a custom project

- **X-MAN™ HaloTag® kits come with:**
  - A vial of the cell line of interest
  - Its parental cell line which can act as a control, and
  - All reagents and buffer necessary to perform a set of experiments.

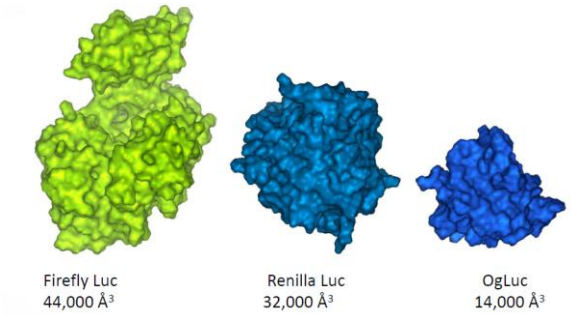
These products are governed by HaloTag® and Horizon Discovery Limited Use Label Licenses

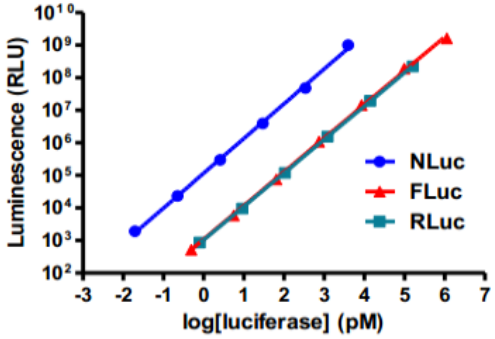
Agenda

- Introduction to Horizon's Technology
- X-MAN™ HaloTag® Reporter Kits
- X-MAN™ NanoLuc™ Reporter Kits

X-MAN™ NanoLuc™ Reporter Lines: Technology Background

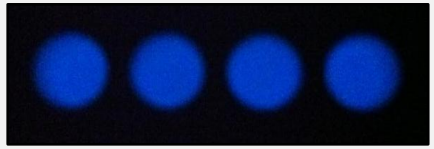
- NanoLuc™ derived from a new luciferase from Deep Ocean Shrimp (Oplophorus)
- Serially mutated to create a monomeric & 150-fold brighter luciferase vs FF and Renilla
- Secreted or intracellular; ATP independent light emission with novel substrates
- **Able to detect gene/protein levels at very low endogenous expression levels**
- Endogenous high-throughput screening now possible





150 fold increased specific activity compared with firefly or Renilla luciferase

Image of live cells expressing NLuc



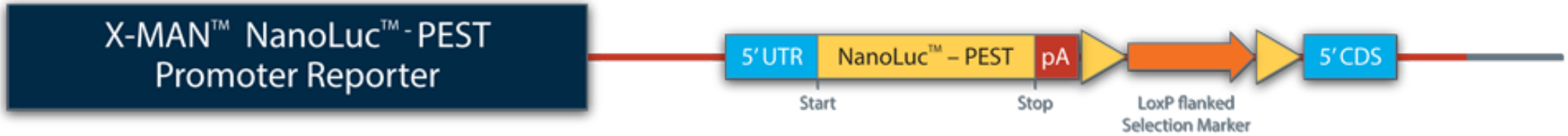
Captured with a handheld iPhone

Better biology … Endogenous HTS … Biologically relevant results

X-MAN™ NanoLuc™ Kit Formats

X-MAN™ NanoLuc™-PEST promoter kit

- Promoter fusions with PEST-NanoLuc™ for rapid turnover and thus dynamic signal kinetics
- Short intracellular lifetime



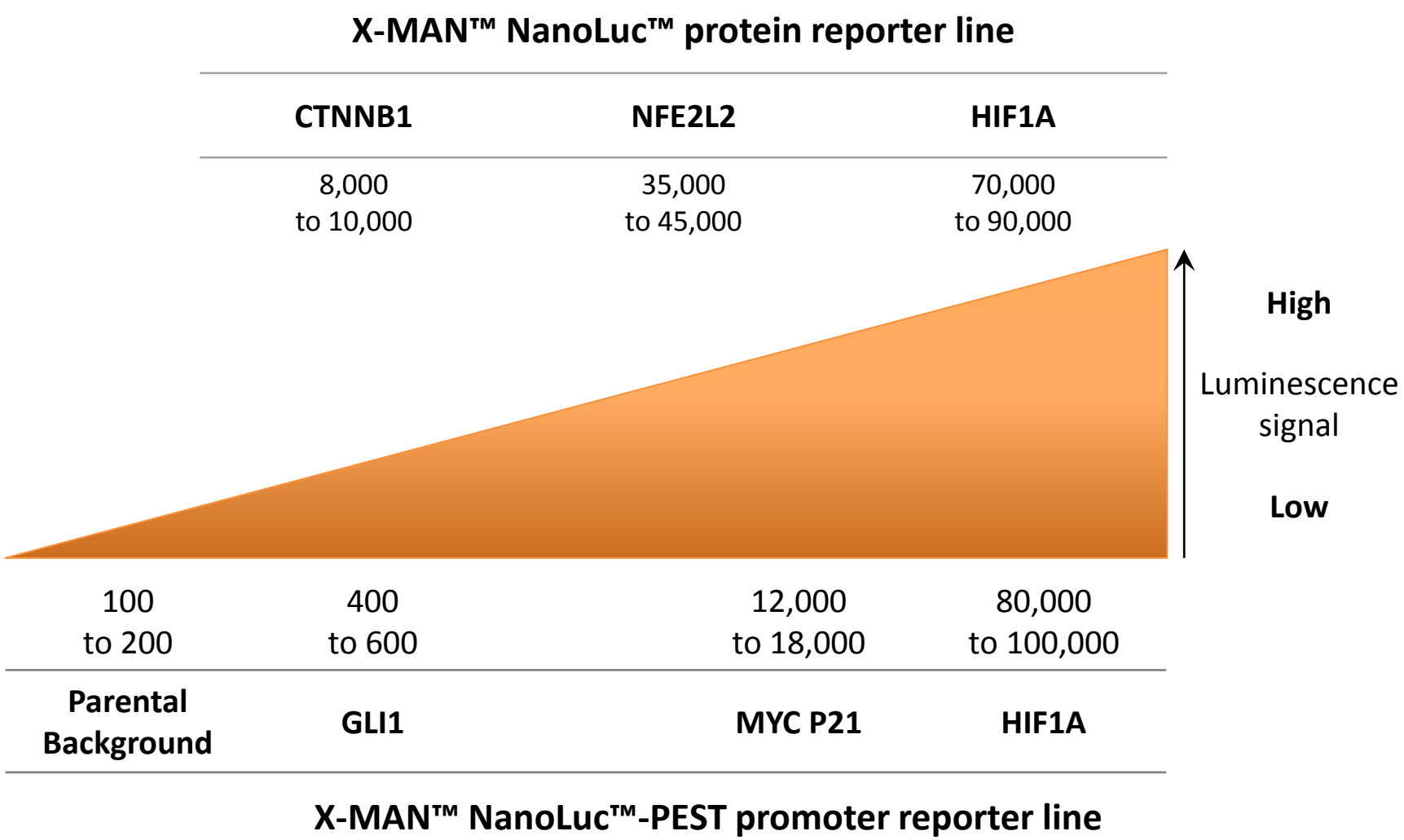
X-MAN™ NanoLuc™ protein kit

- Engineered to generate N- or C-terminal fusion of NanoLuc™ to the endogenous protein of interest
- Ideal for use in protein expression reporter assays

X-MAN™ NanoLuc™ protein reporter control cell lines are also available

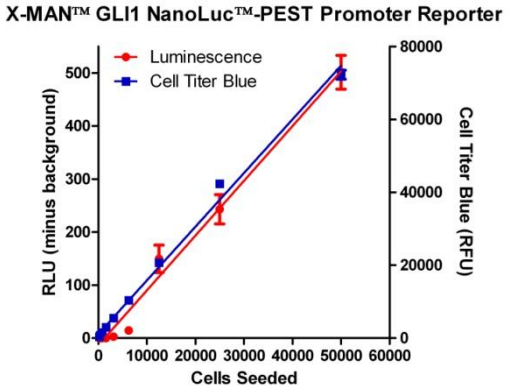
NanoLuc™: Signal Range

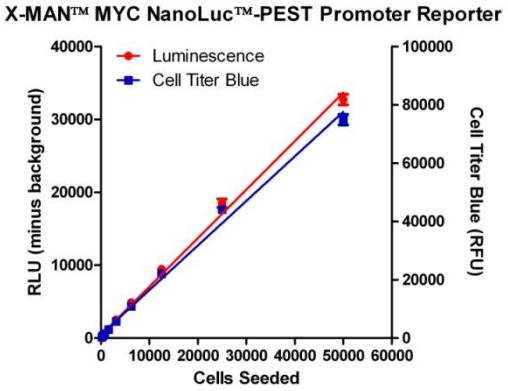
- Baseline luminescence signal is specific for each NanoLuc ™ reporter line, dependent on gene or protein expression levels

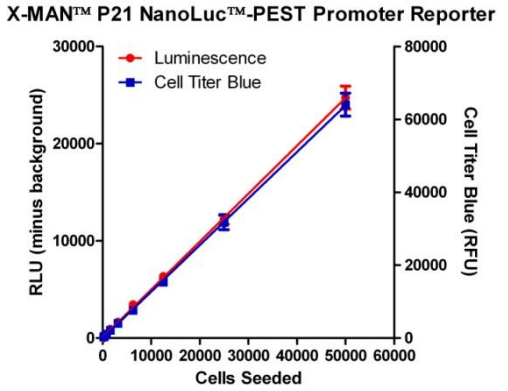


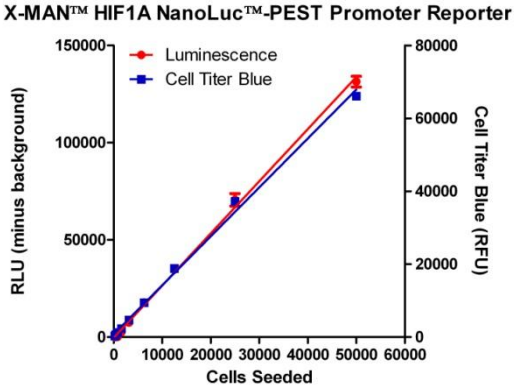
NanoLuc™: Signal Linearity

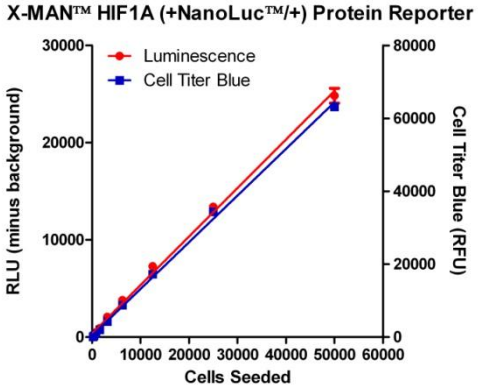
- Good signal linearity with increasing cell number
- Minimal background luciferase signal in parental cells, even at high cell densities

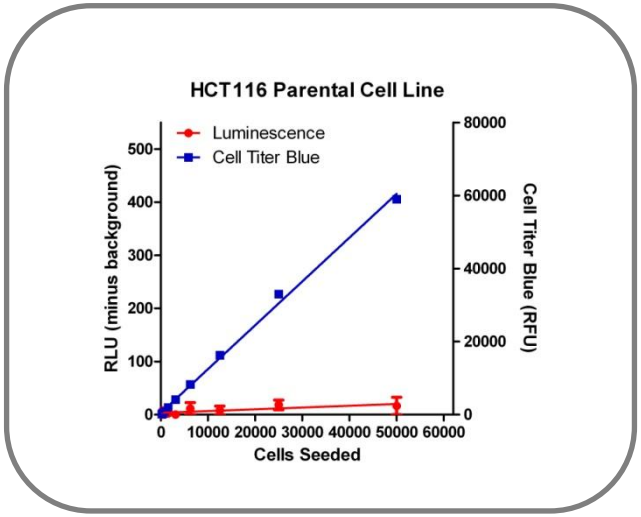








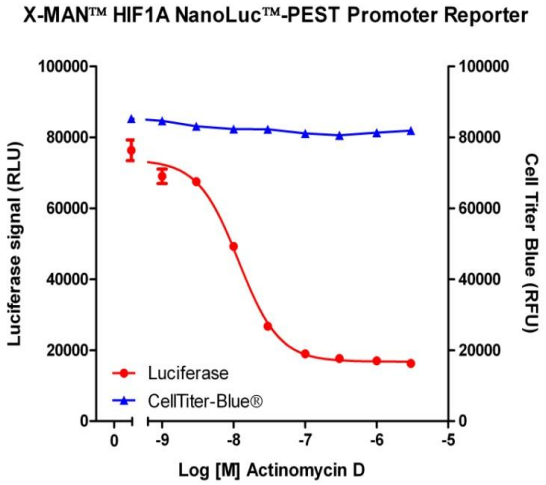


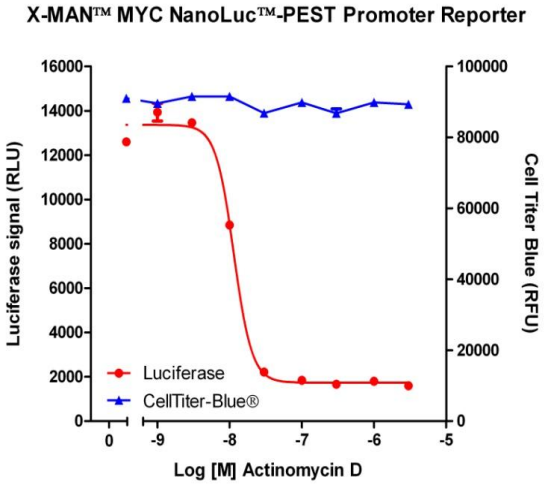


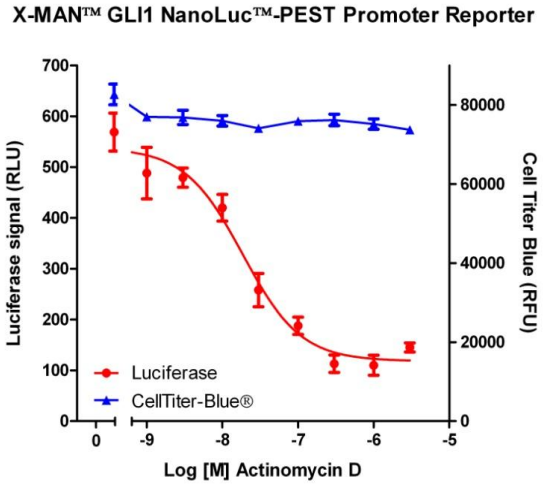
NanoLuc™: Excellent signal dynamics

➢ Dynamics of **promoter reporter** lines:

- 6h treatment with a Actinomycin D
- Rapid and robust decreases in luciferase signal
- Good induction kinetics on inducible genes
- CellTiter-Blue® to control for effects on cell viability





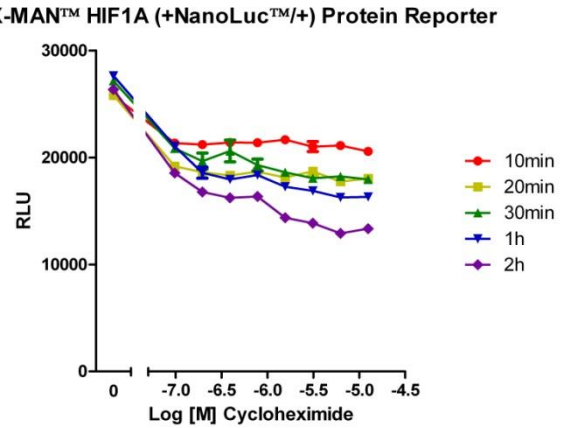


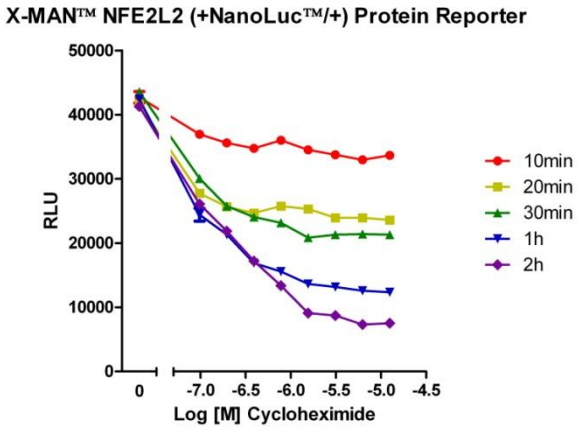
NanoLuc™: Excellent signal dynamics

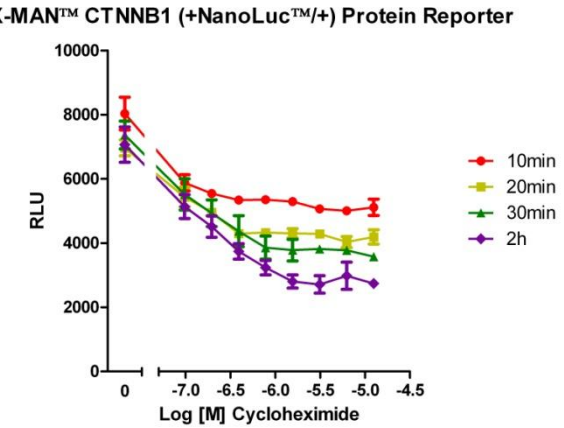
➢ Dynamics of **protein reporter** lines:

- Turnover: Treatment with Cyclohexamide ($t_{1/2}$ dictated by target genes in this scenario)
- Accumulation: 4h treatment with bortezomib
- Good repression and accumulation kinetics of signals generally observed

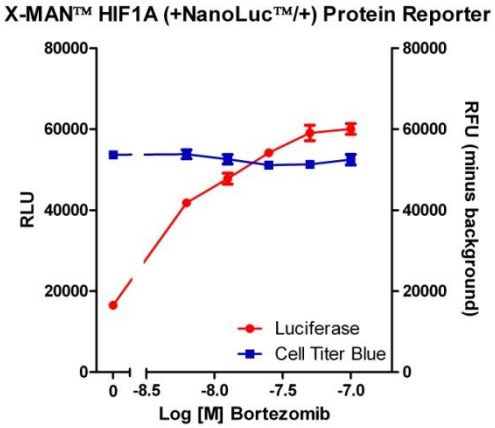
Turnover

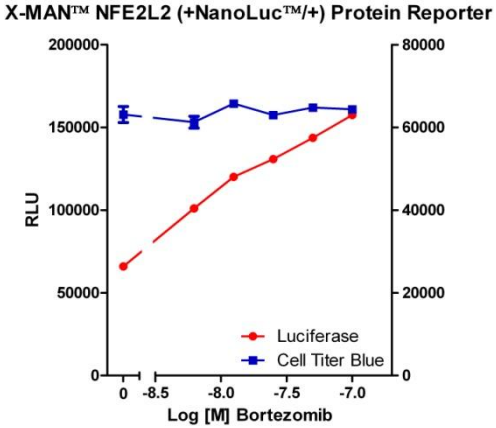


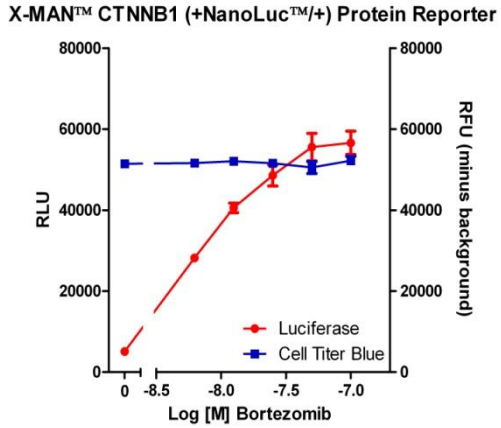




Accumulation







NanoLuc™ Functional Validation: p21 promoter reporter

➢ Monitor modulation of p21 transcription by DNA damaging agents

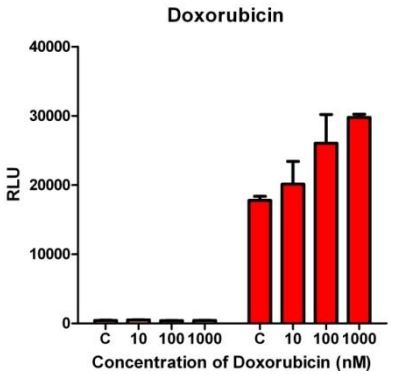
- 6h drug treatment, cell viability measured by multiplexing luciferase assay with CellTiter-Blue®

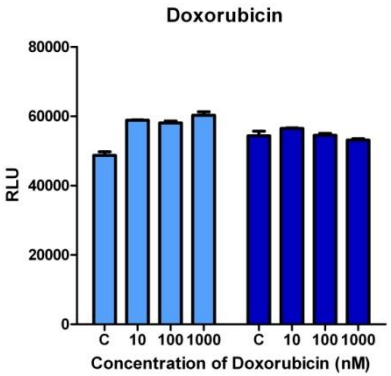
p21 Transcription
(NanoLuc™ activity measurement)

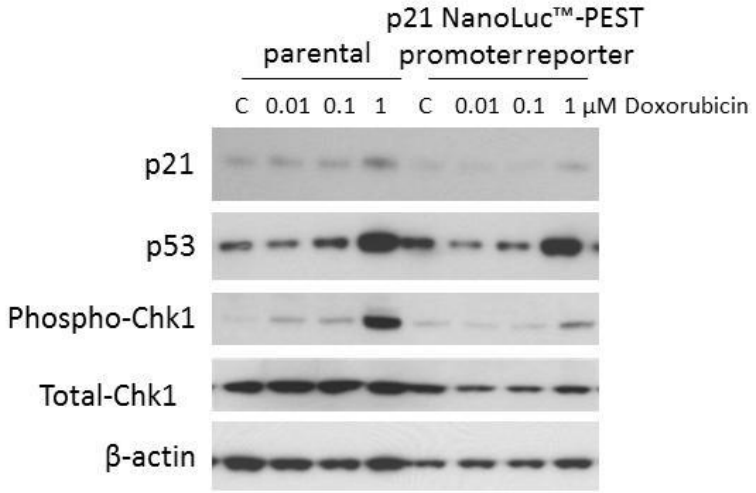
Cell Viability
(measured by CellTiter-Blue®)

Protein Expression

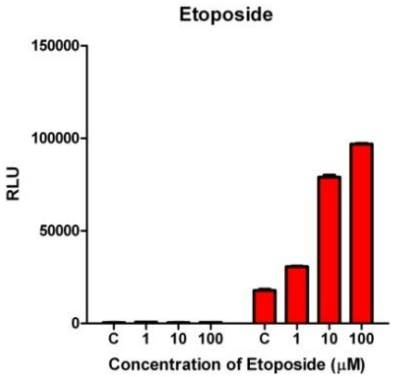
Doxorubicin

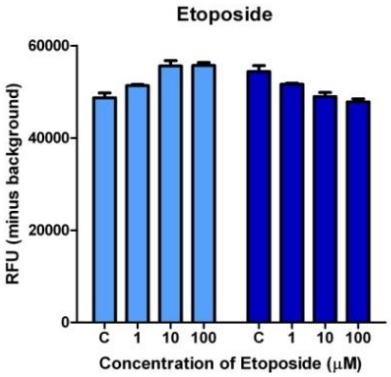


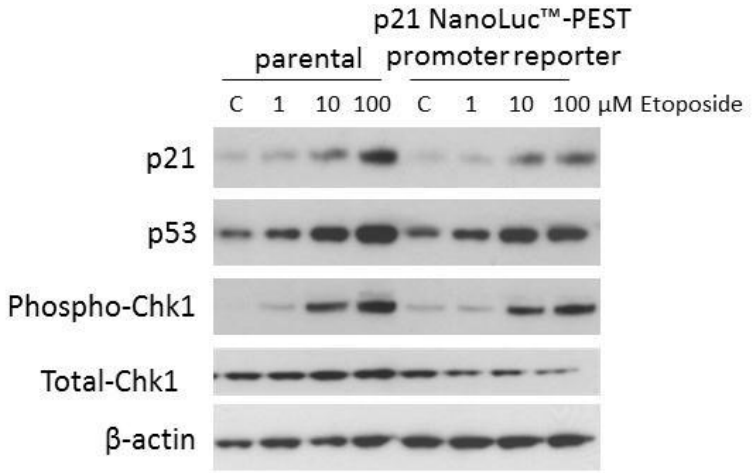




Etoposide







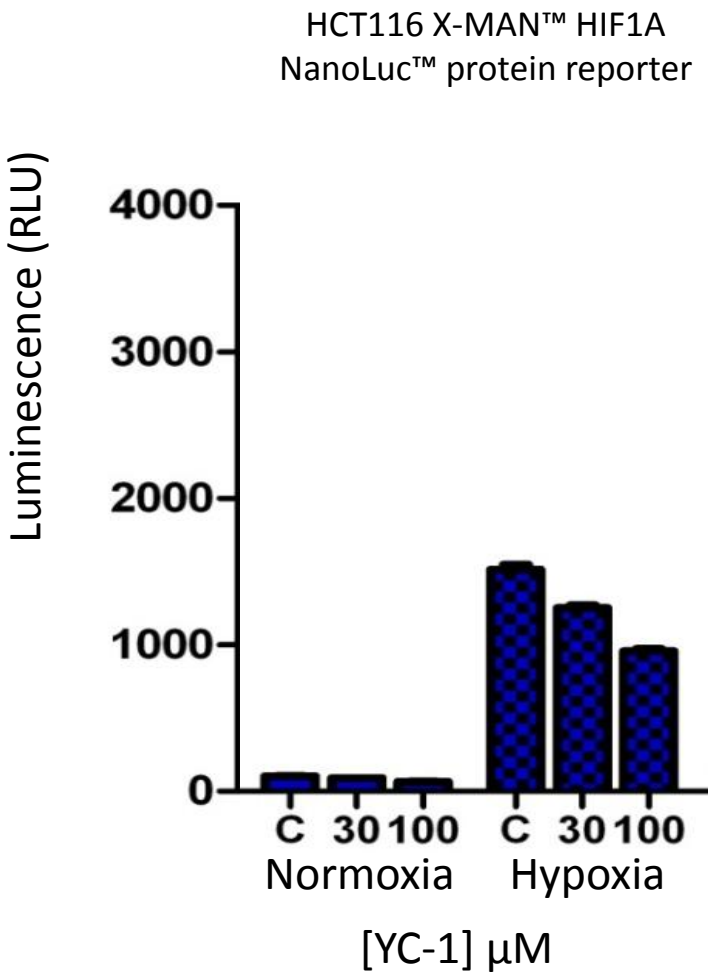
Parental
X-MAN™ p21 NanoLuc™-PEST promoter reporter

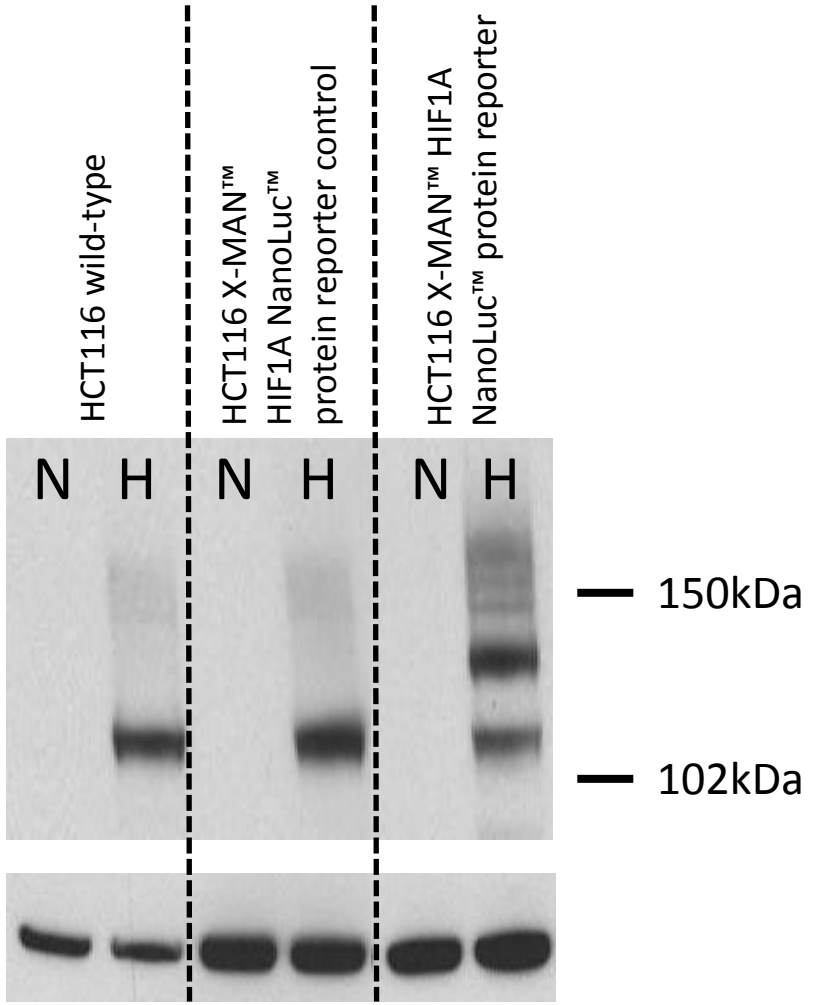
Parental
X-MAN™ p21 NanoLuc™-PEST promoter reporter

NanoLuc™ Functional Validation: HIF1A protein reporter

➢ Monitor induction of HIF1A protein by hypoxia

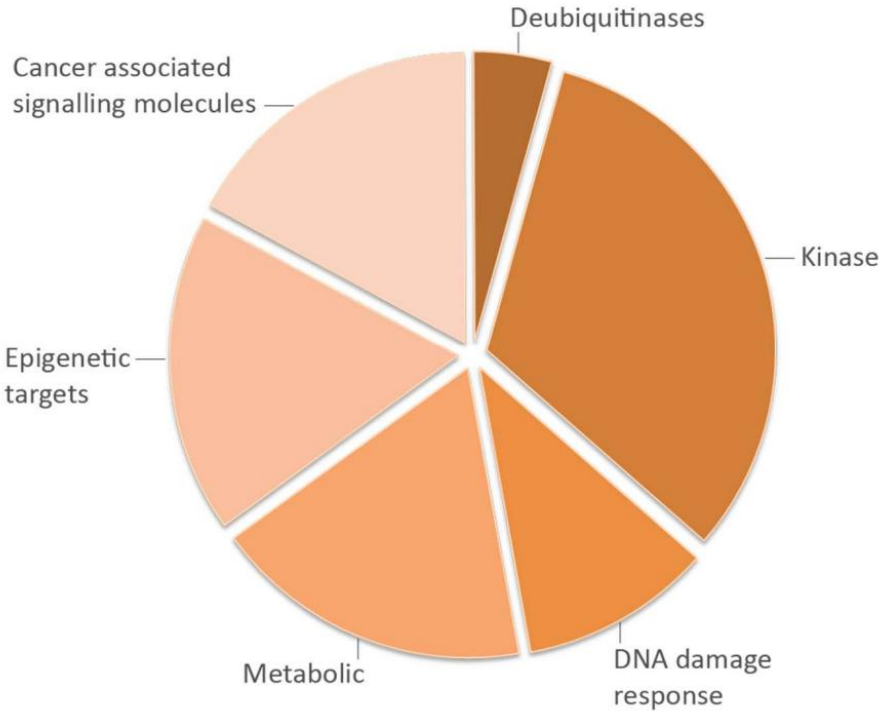
- Natural induction mechanisms preserved – 1% O2 increases the HIF1A fusion protein
- Treatment with YC-1 HIF1A inhibitor decreases expression of HIF1A under hypoxia as determined by NanoLuc™ protein reporter





NanoLuc™ Screening Application: HIF1A protein reporter

- Used in 2 different screening formats
  - siRNA screen at Horizon using our library of over 2000 druggable siRNAs
  - Compound screen at NCATS, using the NCGC Pharmaceutical Collection of approved and investigational drugs

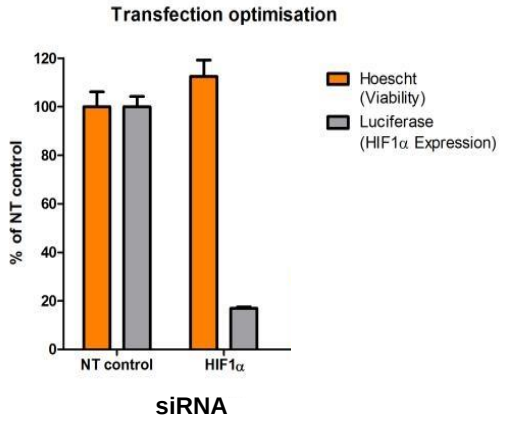


	siRNA Screen	Compound Screen
Plate format	384	1536
Number of Reagents	2235	2514
Z’	0.5	0.7
%CV	6.5%	6.1%

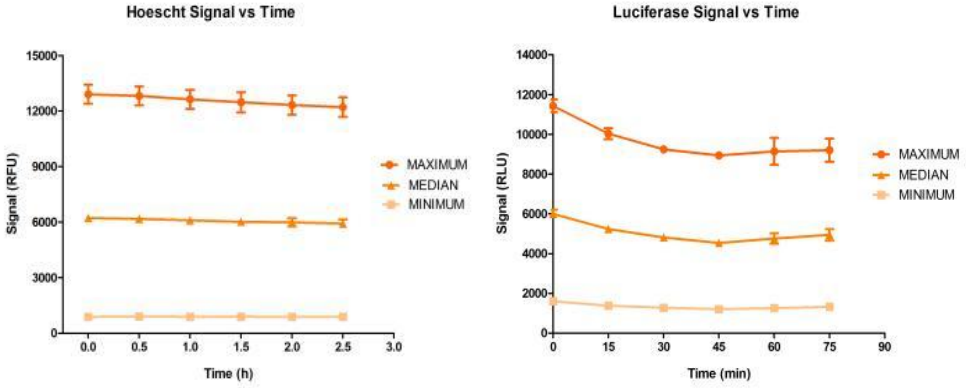
National Center for Advancing Translational Sciences

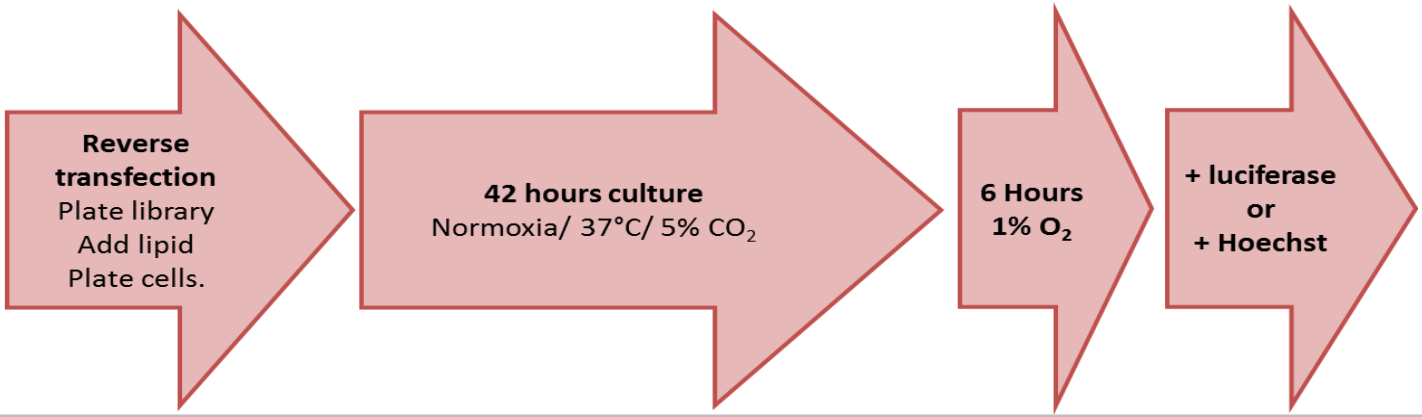
NanoLuc™ Screening Application: HIF1A siRNA Screen

- Transfection conditions were optimised to provide conditions that achieve optimal siRNA knockdown with minimal effects on viability.



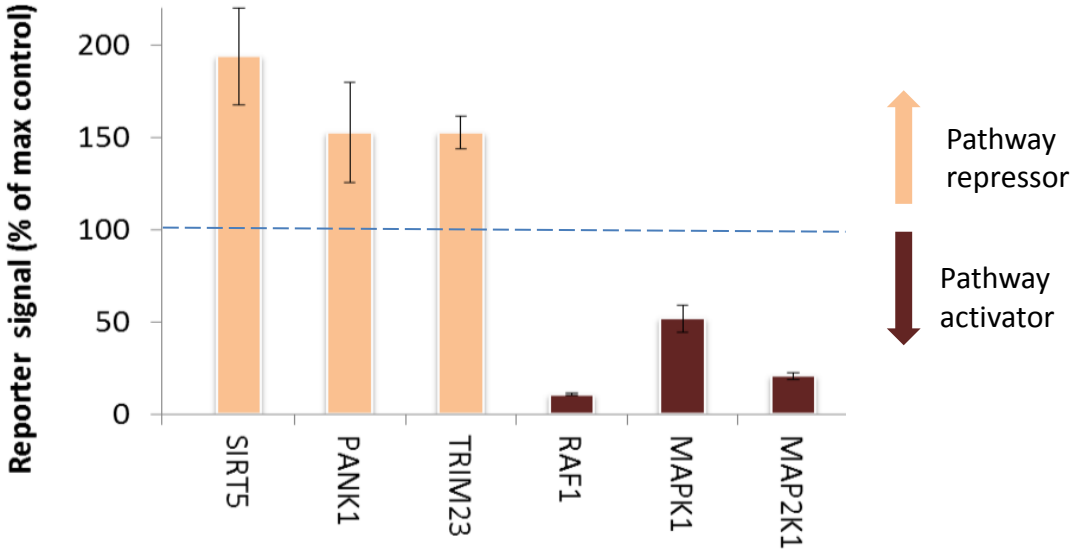
- We concurrently measured:
  - Hoescht nuclear marker to assess viability
  - NanoLuc™ luciferase signal to assess HIF1α protein levels

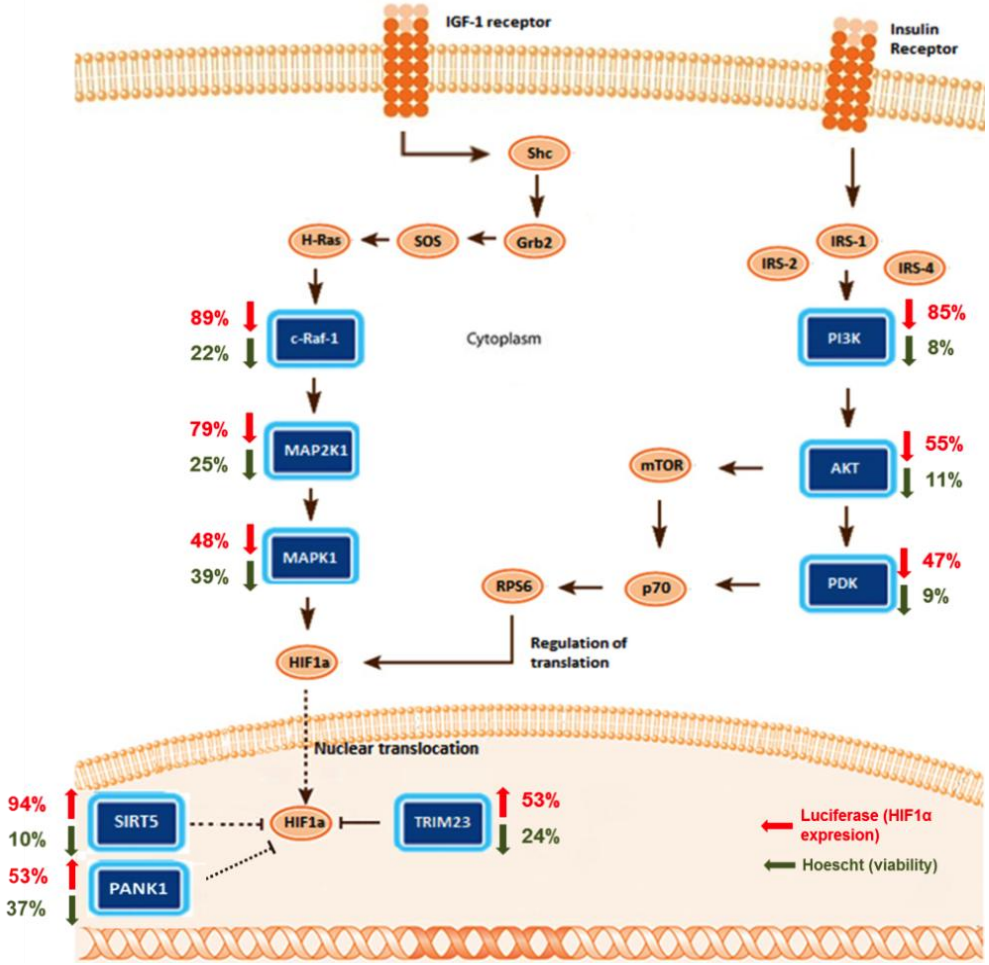




NanoLuc™ Screening Application: HIF1A siRNA Screen

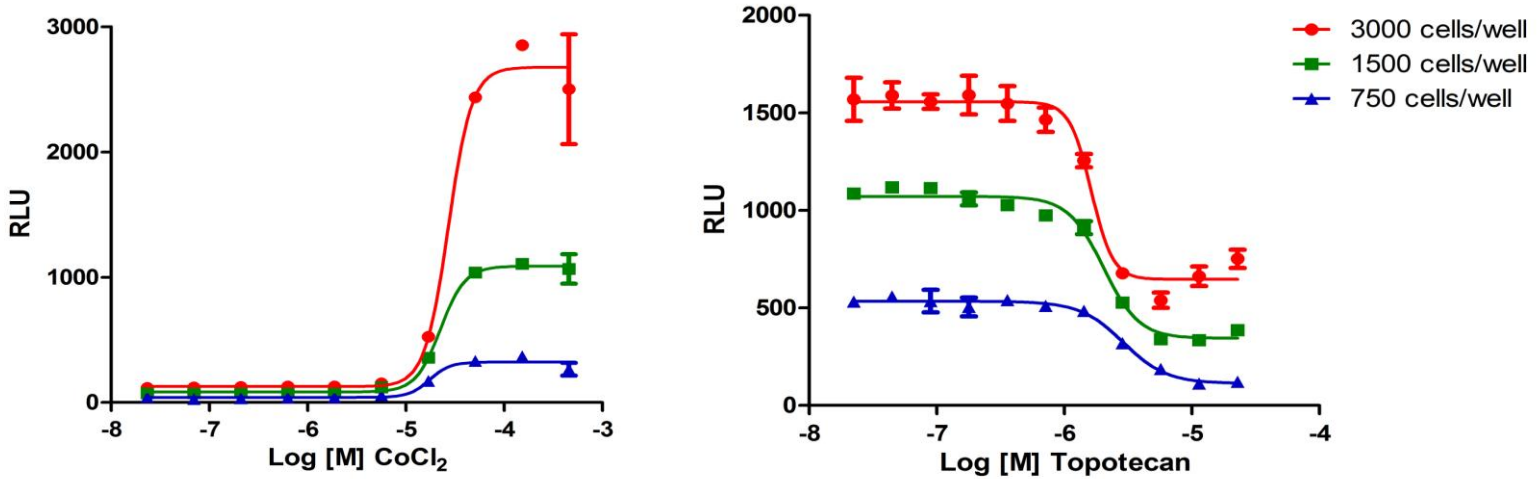
- Subset of kinase, DUB & epigenome hits shown
- Known and novel transcription targets found
- Modulators both activating and repressing HIF1A





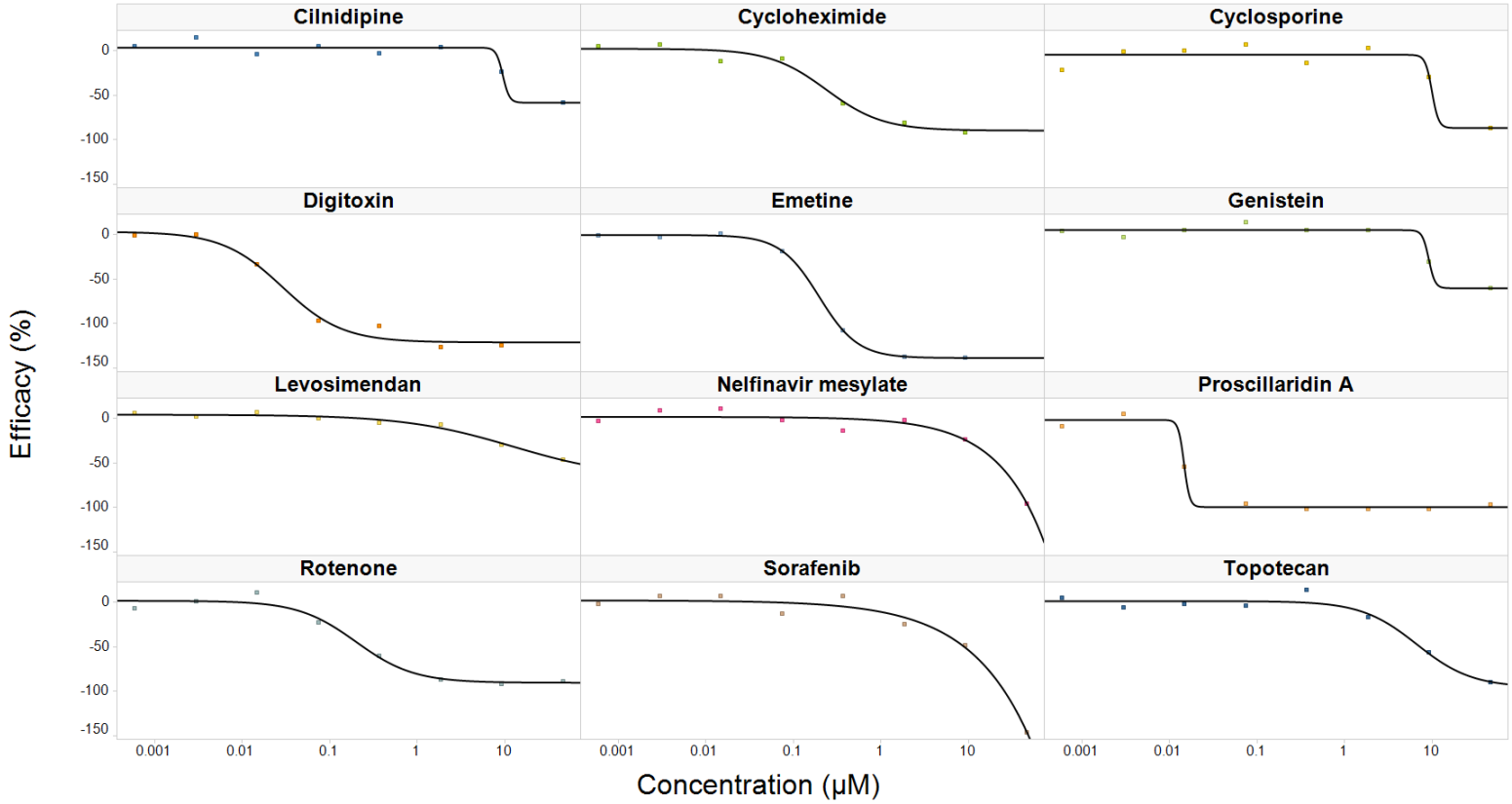
NanoLuc™ Screening Application: NCATS HIF1A Compound Screen

- Cells were seeded into 1536-well plates and induction of HIF1A performed using 1% oxygen or the hypoxic-mimetic $CoCl_2$
  - Knockdown of HIF1A protein reporter signal was confirmed using Topotecan after a 16h drug exposure
  - 1500 cells/well selected for screen, giving a good assay window



NanoLuc™ Screening Application: NCATS HIF1A Compound Screen

- A high-throughput screen was run in 1536-well plate format using the NCGC Pharmaceutical Collection of approved and investigational drugs.
  - 1500 cells per well, 18-hour compound incubation time, 1% O_2
  - 7 concentrations (0.6 nM-46 µM or 1.1 nM-92 µM), duplicate runs
  - The assay performed well returning strong hits for known regulators
  - QC parameters were good with Z' of 0.70 and CV of 6.1%.



X-MAN™ NanoLuc™ Kits Currently Available

NCBI Gene Name	Common name	X-MAN™ NanoLuc™-PEST promoter reporter	X-MAN™ (+NanoLuc™/+) protein reporter	X-MAN™ NanoLuc™ protein reporter control
HIF1A	HIF1α	x	x	x
NFE2L2	NRF2	---	x	x
CTNNB1	β-CATENIN	---	x	x
CDKN1A	p21, CIP1, $P21^{CIP1}$	x	---	---
MYC	c-MYC	x	---	---
GLI1	GLI	x	---	---

- Additional lines are constantly being developed by Horizon or can be developed as a custom project
- **X-MAN™ NanoLuc™ kits are supplied with:**
  - A vial of the cell line of interest
  - All reagents and buffer necessary to perform a set of experiments
- Kits are available in 10, 100, 500, 1,000 ml and custom reporter volume sizes
- **These products are governed by NanoLuc™ and Horizon Discovery Limited Use Label Licenses**

Selection of Customers / Collaborators (>650 unique partners)

INDUSTRY

ACADEMIA

horizon

precision genome editing

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