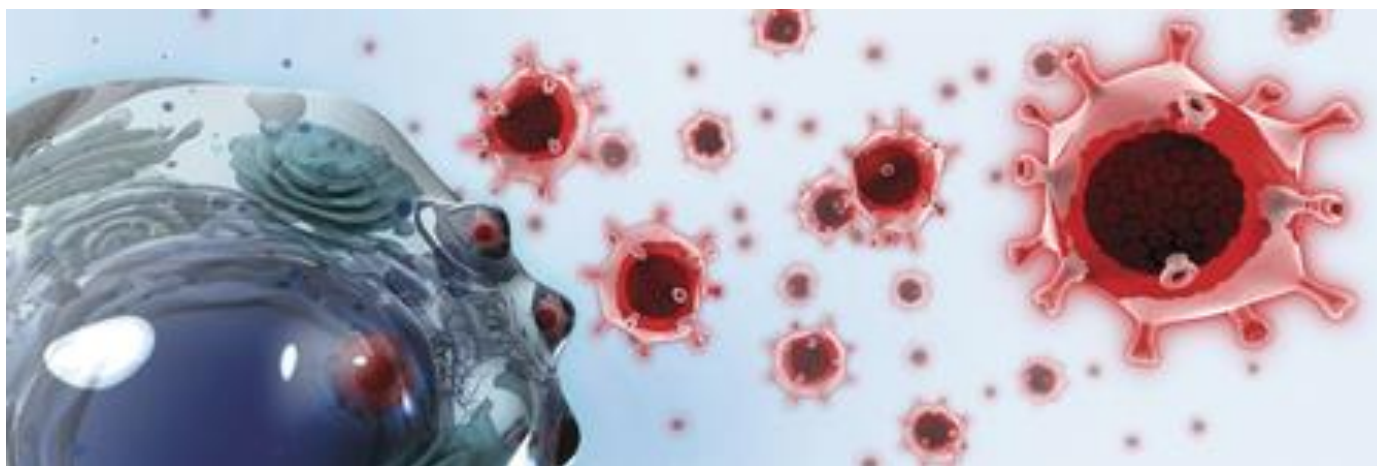
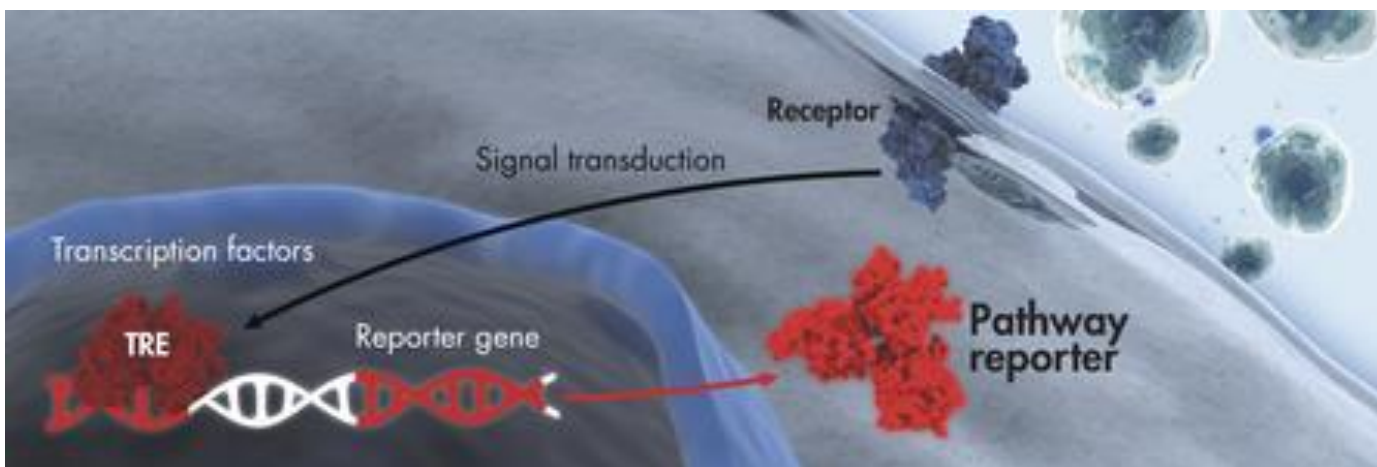
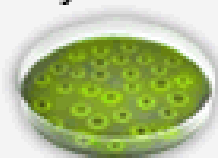




Contact: Anisha Kharkia; anisha.kharkia@qiagen.com

Functional analysis of genes, biologics and small molecule compounds

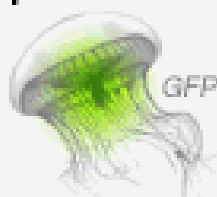
Analysis with Cell-Based Reporters



stable cell lines



luciferase

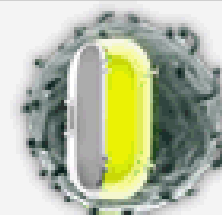


GFP



lentiviral delivery

*multi-pathway
analysis*



transfection / transduction

The Signal Reporter Assays are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

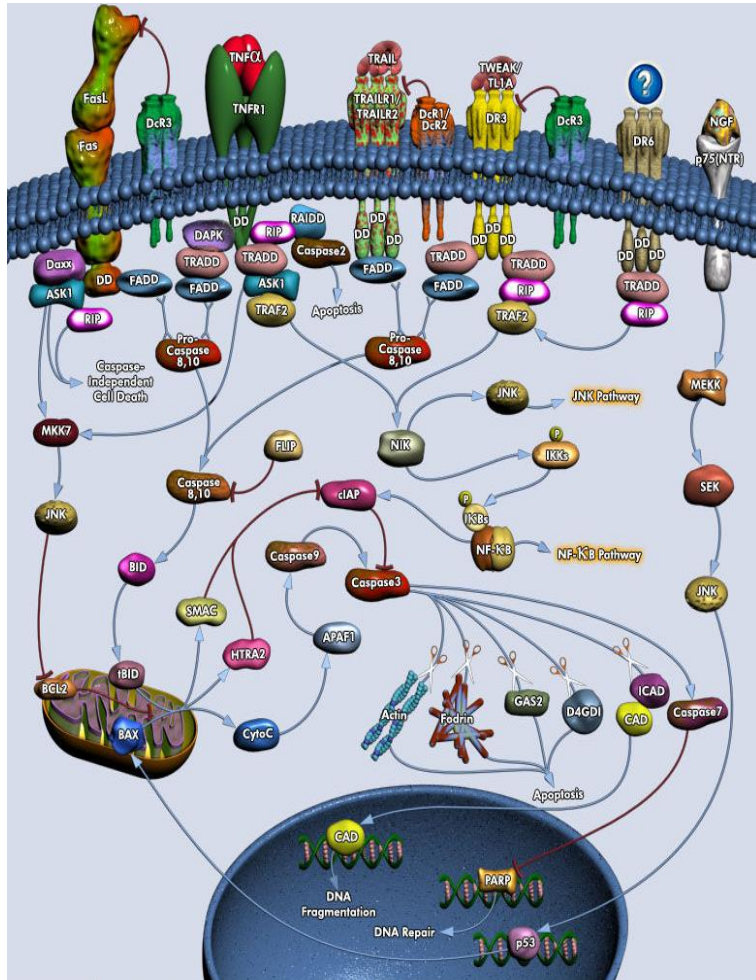


Image reference: [Apoptosis through death receptors. Pathway central, GeneGlobe.](#)

- 1 [Taking on challenges with reporter and lenti assays](#)
- 2 [How Signal Reporter and Lenti Assays work](#)
- 3 [How you can use reporter assays in your research](#)
- 4 [Summary](#)

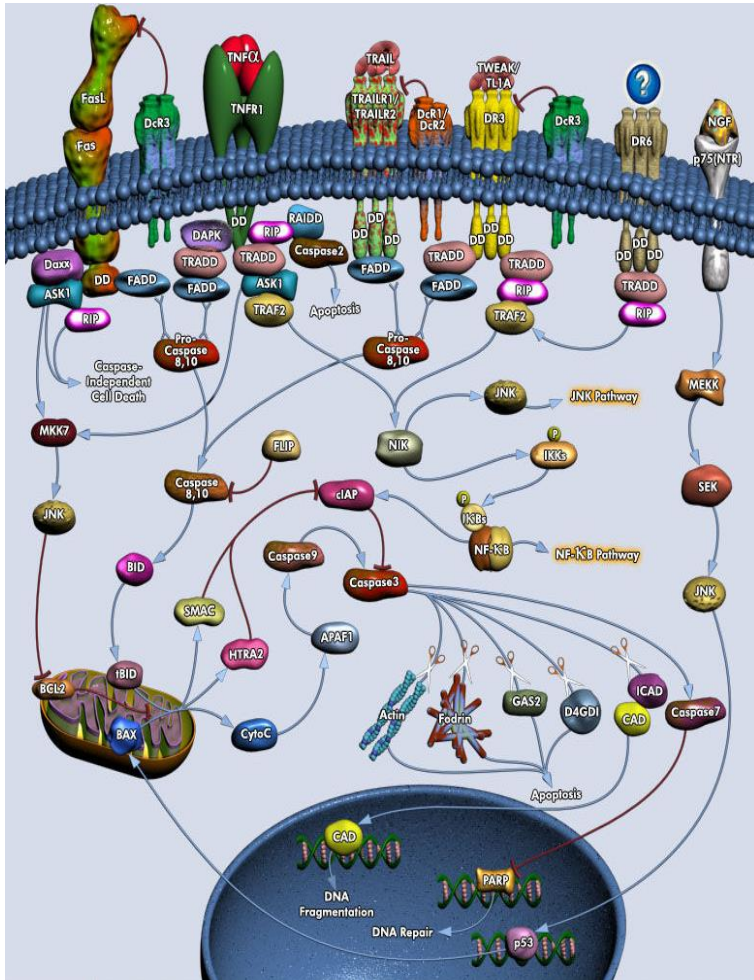


Image reference: [Apoptosis through death receptors. Pathway central, GeneGlobe.](#)

1 Taking on challenges with reporter and lenti assays

a Challenges associated

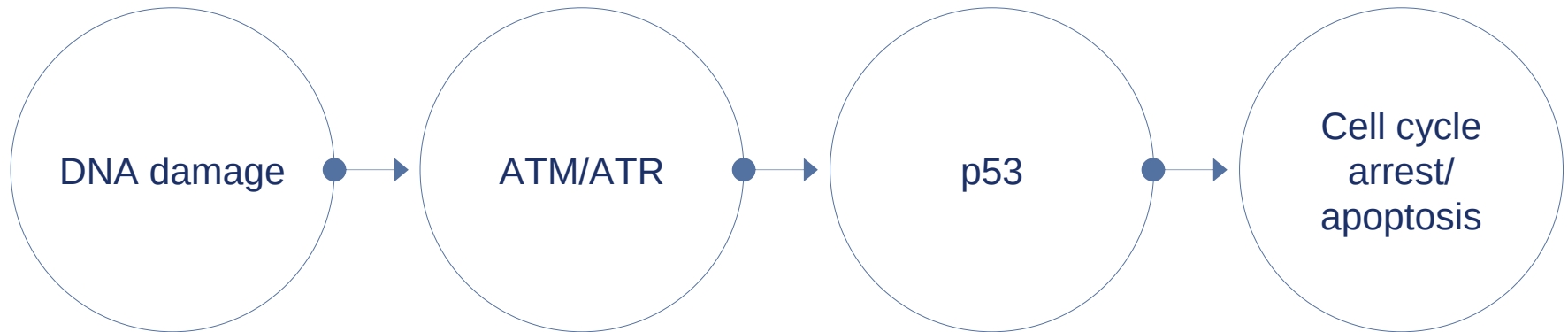
b Solution: Signal Reporter and Lenti Assay

2 How Signal Reporter and Lenti Assays work

3 How you can use Signal Reporter Assays in your research

4 Summary

Moving from a signaling flowchart perspective
to a signaling network perspective



Basic signal transduction flowchart

Intra-experiment variability

- Well-to-well, plate-to-plate reproducibility

Inter-experiment variability

- “Status” of the cells
- Who in the lab is conducting the experiments

Assay performance

- Sensitivity, specificity, signal-to-noise ratio and simplicity

Frustration of analyzing one pathway at a time

- Time consuming
- Misleading

Studies limited to cells that are easily transfected

- What about primary cells?
- What about stem cells?
- What about cell lines that are difficult to transfect?

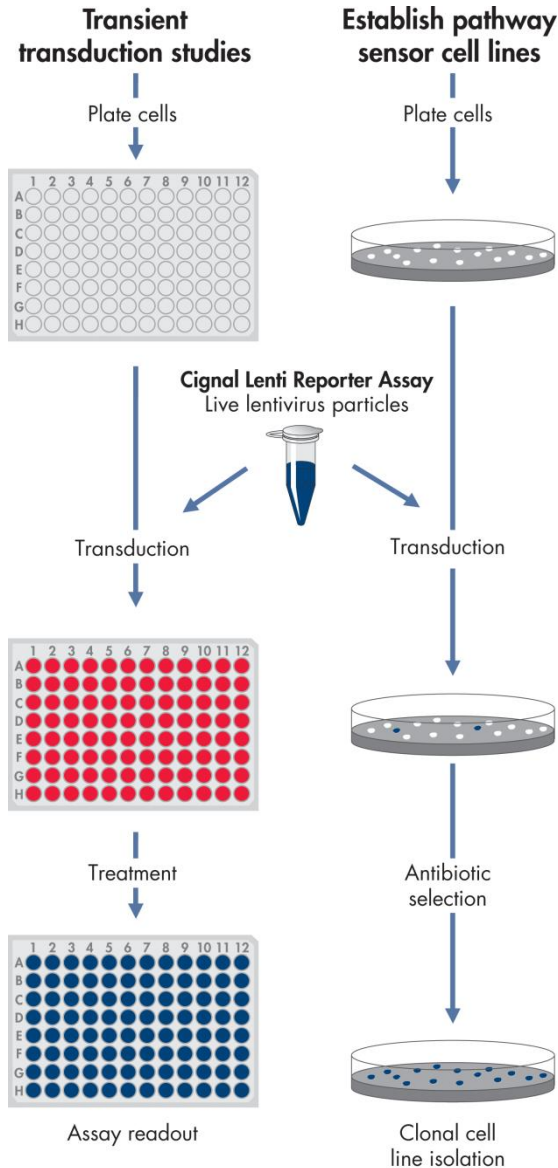
Engineered and formatted for superior performance

- Custom engineered transcriptional response elements (TRE)
- Use reporter genes with outstanding sensitivity and specificity
- Internal, positive and negative controls for reproducible results
- Luciferase and GFP formats are available
- Lentiviral particles are ready for transduction right out of the box

Signal Finder 10-Pathway Arrays

- Enable the rapid and reliable study of multiple signaling pathways

Signal Lenti Reporters: benefits



Transduce any mammalian cell

- Primary cells
- Stem cells
- Difficult-to-transfect cell lines

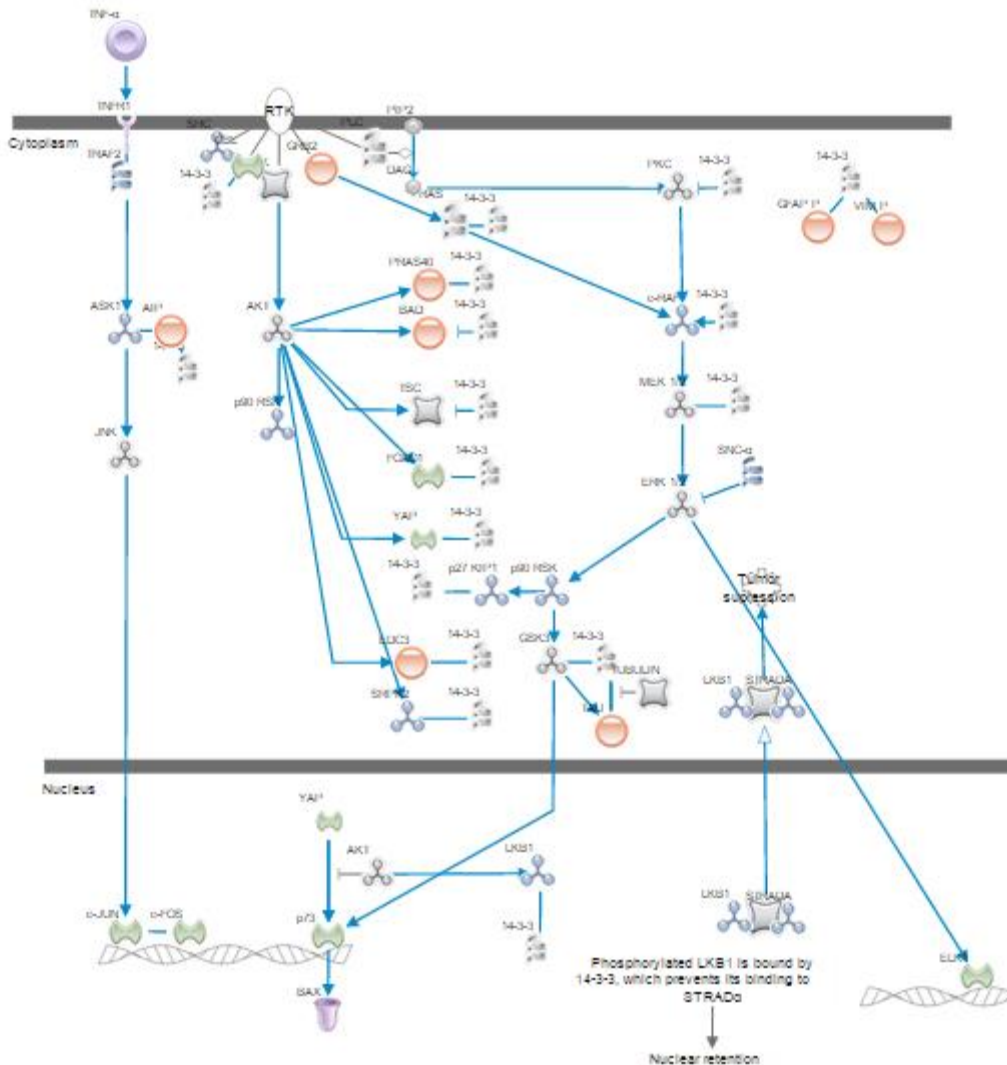
Transduction-ready

- No lentiviral generation
- No titering assays

Versatile system

- Transient experiments
- Generate pathway sensor cell lines

Research challenge: constructing signaling networks



We found 4204 Interaction Results Page 1 ...

MAPK1 (kinase)

mitogen-activated protein kin...

Synonyms:
9030612K14Rik, AA407128, AU018647, BcDNA:RE08694, C7827...

Entrez Gene ID:
Human (MAPK1 EG:5594), Mouse (Mapk1 EG:26413), Rat (Map...

RELA (transcription r...

v-rel avian reticuloendotheli...

Synonyms:
anon-EST:GressD7, CG6667, dl, Dmel CG6667, Dor, Dorsal,...

Entrez Gene ID:
Human (RELA EG:5970), Mouse (Rela EG:19697), Rat (Rela ...

AKT1 (kinase)

v-akt murine thymoma viral on...

Synonyms:
AKT, AKT1, akt2l, CWS6, LOC100331515, PKB, PKB-ALPHA, P...

Entrez Gene ID:
Human (AKT1 EG:207), Mouse (Akt1 EG:11651), Rat (Akt1 E...

MAPK8 (kinase)

mitogen-activated protein kin...

Synonyms:
A1849689, basket, bsk, Bsk1, C-JUN N-TERMINAL KINASE1, ...

Entrez Gene ID:
Human (MAPK8 EG:5599), Mouse (Mapk8 EG:26419), Rat (Map...

RAF1 (kinase)

Raf-1 proto-oncogene, serine/...

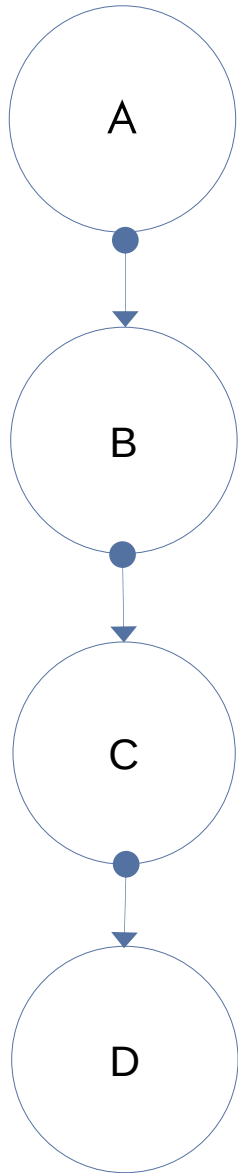
Synonyms:

NFKBIA (transcription...

nuclear factor of kappa light...

Image reference: [TNF pathway and interaction network. GeneGlobe](#)

Research challenge: constructing signaling networks



Signal
transduction
cascade
(~40 proteins)

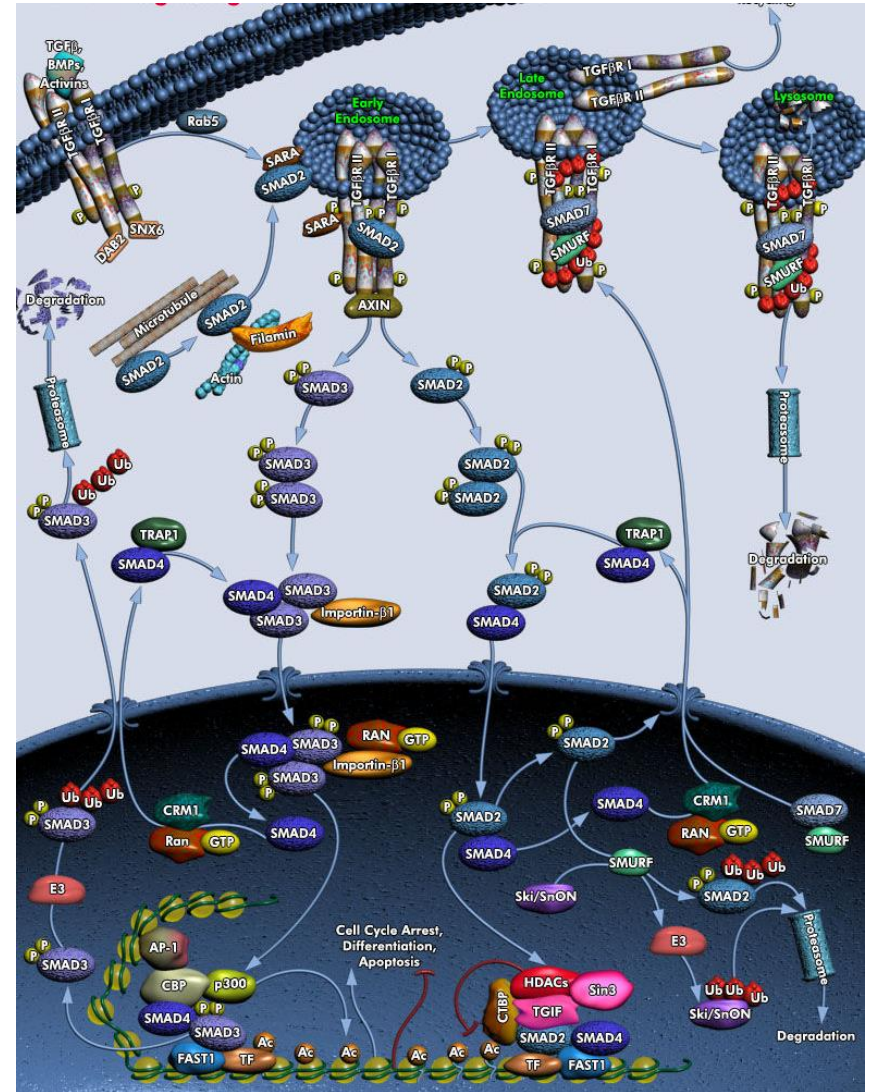


Image reference: [SMAD signaling network](#)

Flexible, pre-optimized solution for cell based assays:

- Functionally validated dual-reporter formulation (luciferase)
 - Minimizes experimental variability, increasing biological relevance
- Ready-to-use reporters and controls, in transfection ready format
 - Enables simple, rapid analysis of the regulation of 45 signal transduction pathways
- Genetically engineered transcriptional regulatory elements (TREs) and destabilized, codon-optimized firefly luciferase
 - Increases the signal to noise ratio, maximizes assay sensitivity and specificity

Available in both luciferase and GFP formats

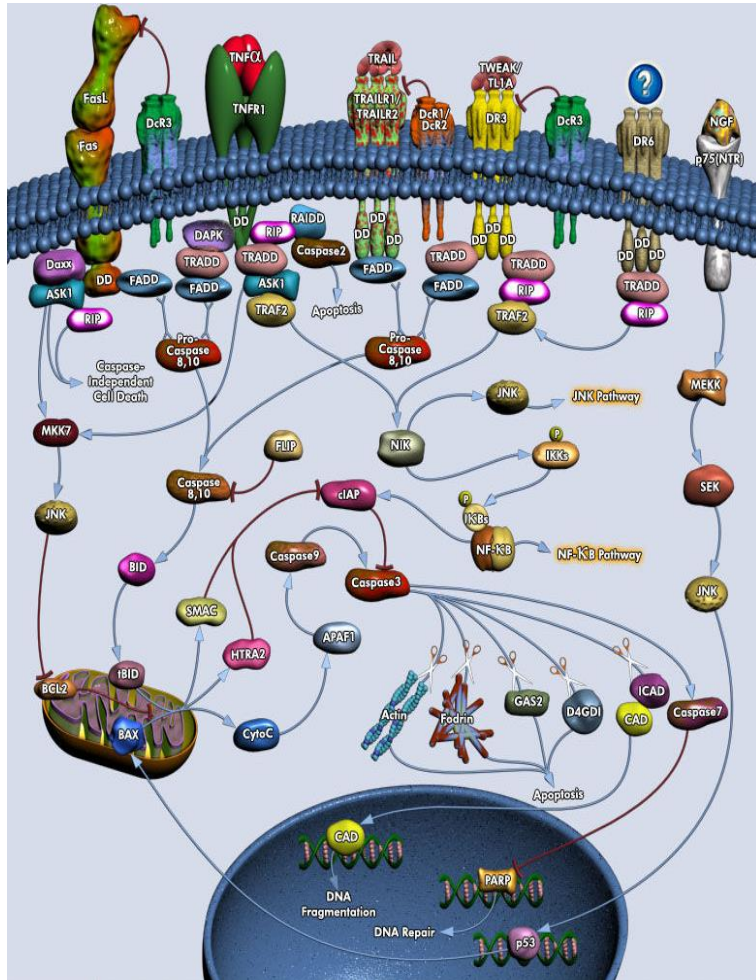


Image reference: [Apoptosis through death receptors. Pathway central, GeneGlobe.](#)

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- 2 [How Signal Reporter and Lenti Assays work](#)
- 3 [How you can use Signal Reporter Assays in your research](#)
- 4 [Summary](#)

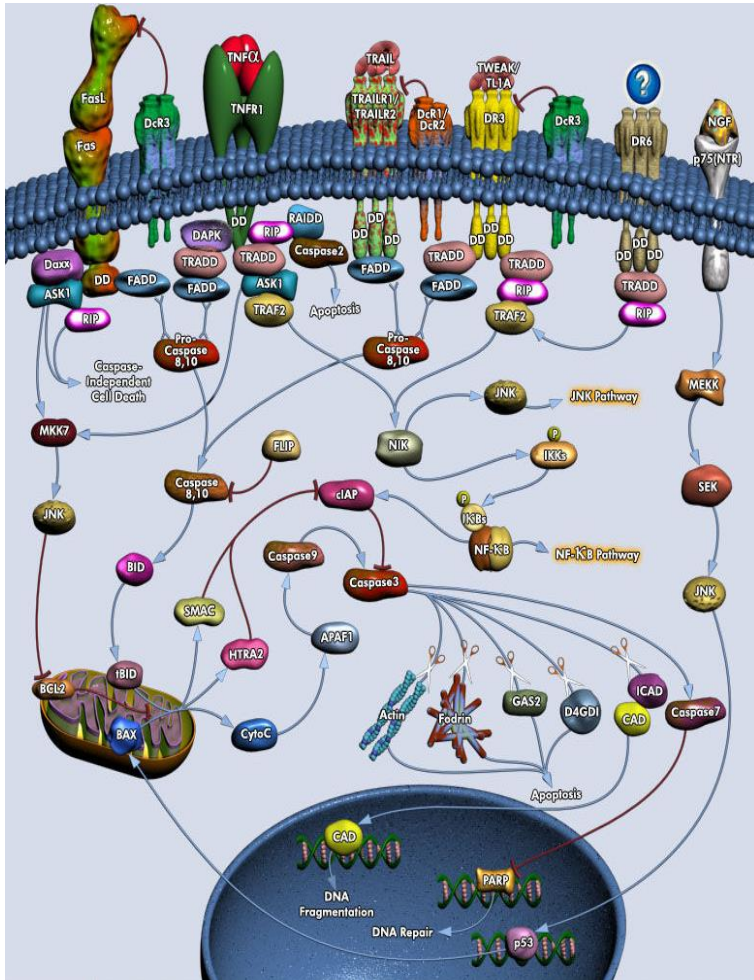


Image reference: [Apoptosis through death receptors. Pathway central, GeneGlobe.](#)

1	Taking on challenges with Signal Reporter Assays								
2	How Signal Reporter and Lenti Assays work <table> <tr> <td>a</td><td>Complete product portfolio</td></tr> <tr> <td>b</td><td>Dual-luciferase assays</td></tr> <tr> <td>a</td><td>High-performance luciferase</td></tr> <tr> <td>b</td><td>Pre-optimized TREs</td></tr> </table>	a	Complete product portfolio	b	Dual-luciferase assays	a	High-performance luciferase	b	Pre-optimized TREs
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3	How you can use Signal Reporter Assays in your research								
4	Summary								

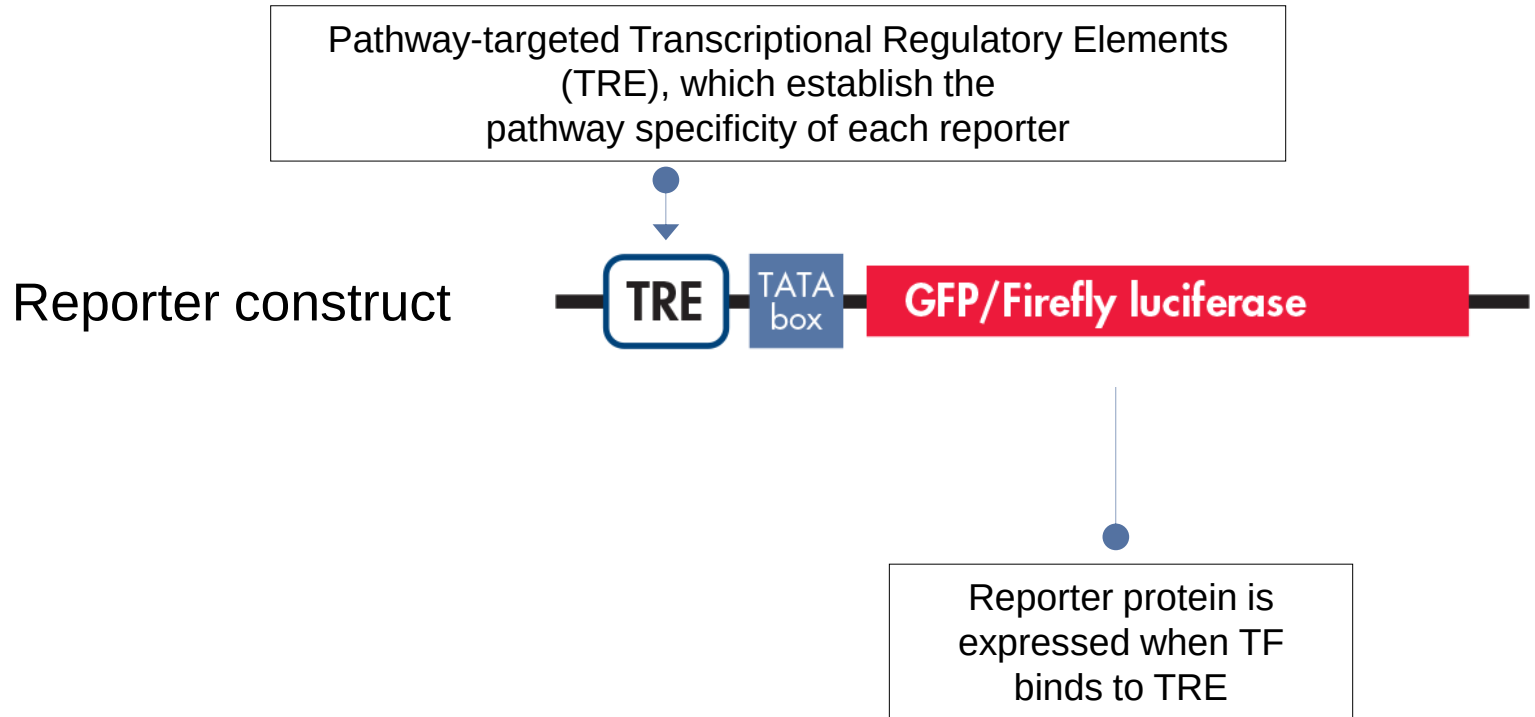
Dual-luciferase format

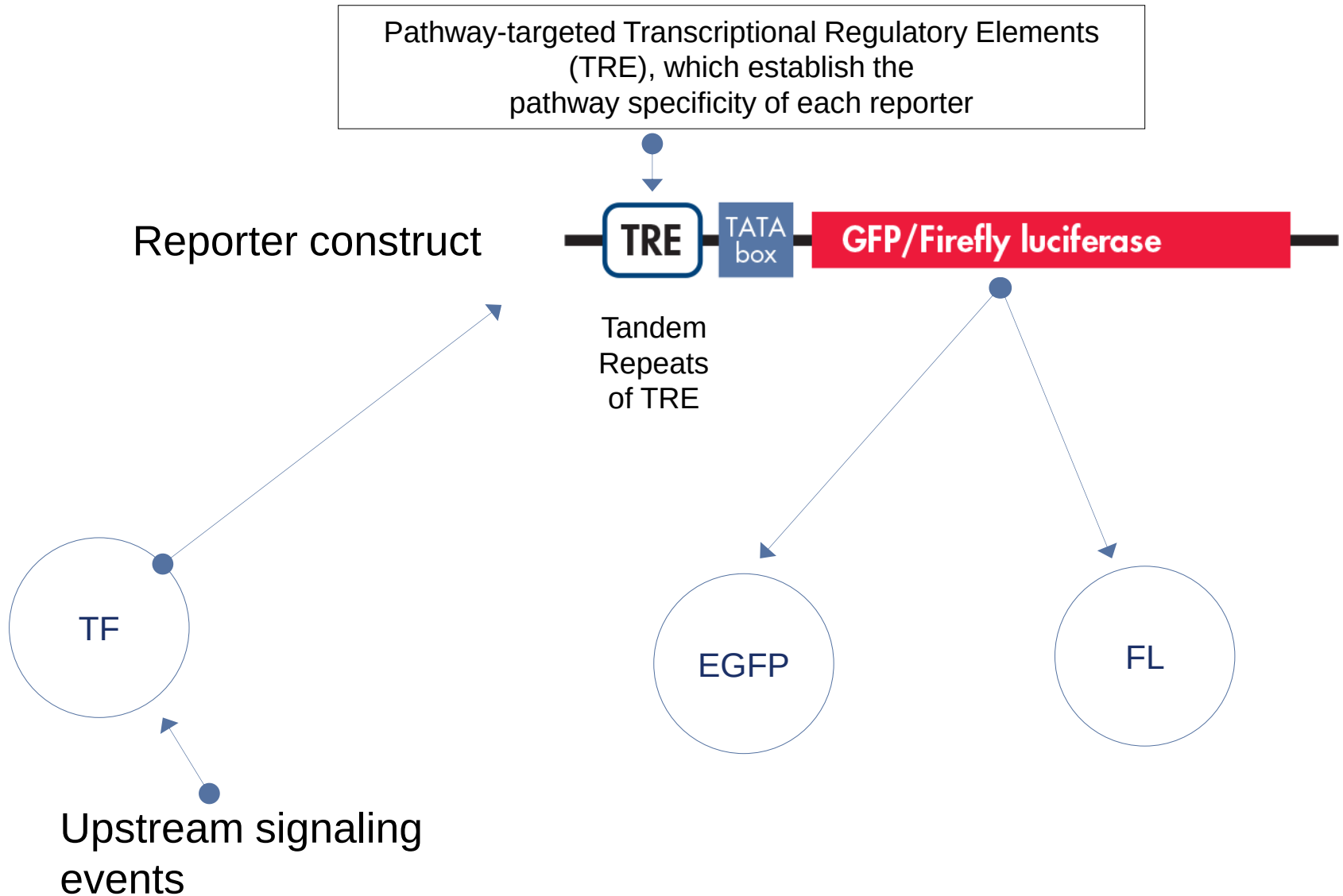
- Quantitative end-point luminescence assay
- Exceptional reproducibility, sensitivity and signal-to-noise ratio
- Average expression from a population of cells

GFP format

- Dynamic live cell assay
- Single cell resolution
- Readout flexibility

(flow cytometry, fluorescent microscopy, fluorometry)





Pathway-targeted Transcriptional Regulatory Elements (TRE), which establish the pathway specificity of each reporter

Reporter construct



Rigorously optimized, functionally validated and ready-to-use reporters

Negative control construct



Positive control construct



Internal control construct



Multiple reporter formats: complete dual Luciferase system

Pathway-targeted Transcriptional Regulatory Elements (TRE), which establish the pathway specificity of each reporter

Reporter construct



Negative control construct



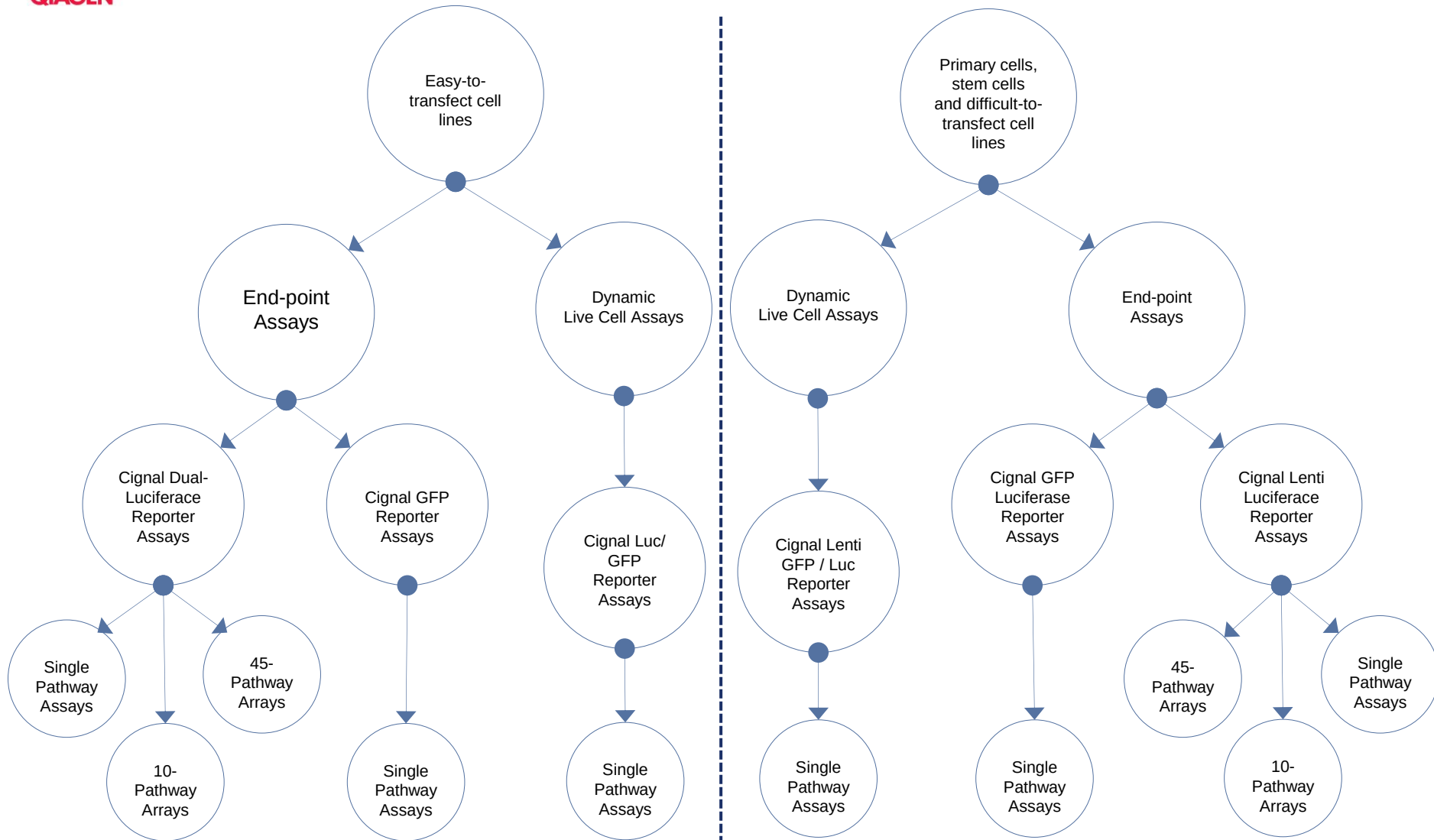
Positive control construct



Internal control construct



Signal Reporter and Lenti Reporter Assays: product offering



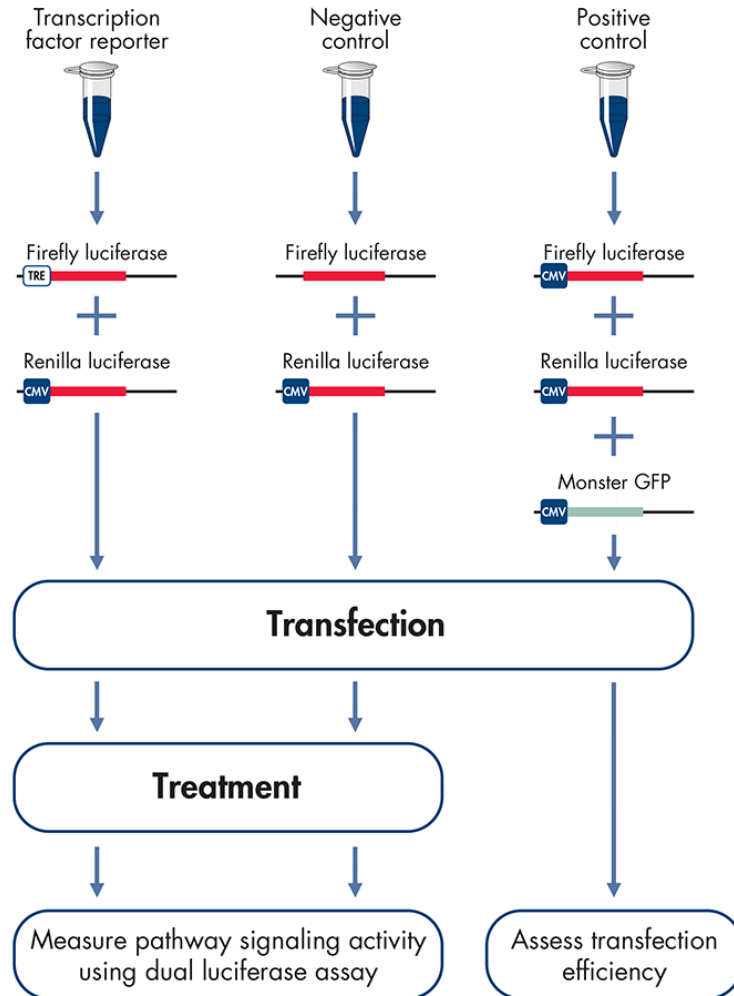
Multiple options to suit your research needs:

plasmid and lentiviral, luciferase and GFP reporters, single, 10-pathway, 45-pathway

Pathway	Transcription Factor	Pathway	Transcription Factor
Amino Acid Deprivation	ATF4/ATF3/ATF2	MAPK/ERK	Elk-1/SRF
Androgen	Androgen Receptor	MAPK/JNK	AP-1
Antioxidant Response	Nrf2 & Nrf1	MEF2	MEF2
ATF6	ATF6	c-myc	Myc/Max
C/EBP	C/EBP	Nanog	Nanog
cAMP/PKA	CREB	NFκB	NFκB
Cell Cycle	E2F/DP1	Notch	RBP-Jκ
DNA Damage	p53	Oct4	Oct4
EGR1	EGR1	Pax6	Pax6
ER Stress	CBF/NF-Y/YY1	PI3K/AKT	FOXO
Estrogen	ER	PKC/Ca ⁺⁺	NFAT
GATA	GATA	PPAR	PPAR
Glucocorticoid	GR	Progesterone	PR
Heat Shock Response	HSF	Retinoic Acid	RAR
Heavy Metal Stress	MTF1	Retinoid X	RXR
Hedgehog	Gli	Sox2	Sox2
HNF4	HNF4	SP1	SP1
Hypoxia	HIF-1	STAT3	STAT3
Interferon Regulation	IRF-1	TGFβ	SMAD2/3/4
Type I Interferon	STAT1/STAT2	VDRE	Vitamin D Receptor
Interferon Gamma	STAT1/STAT1	Wnt	TCF/LEF
KLF4	KLF4	Xenobiotic	AhR
Liver X	LXR		

Pathway	Transcription Factor	Pathway	Transcription Factor
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cAMP/PKA	CREB	NFκB	NFκB
Cell Cycle	E2F/DP1	Notch	RBP-Jκ
DNA Damage	p53	Oct4	Oct4
EGR1	EGR1	Pax6	Pax6
ER Stress	CBF/NF-Y/YY1	PI3K/AKT	FOXO
Estrogen	ER	PKC/Ca ⁺⁺	NFAT
GATA	GATA	PPAR	PPAR
Glucocorticoid	GR	Progesterone	PR
Heat Shock Response	HSF	Retinoic Acid	RAR
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Liver X	LXR		

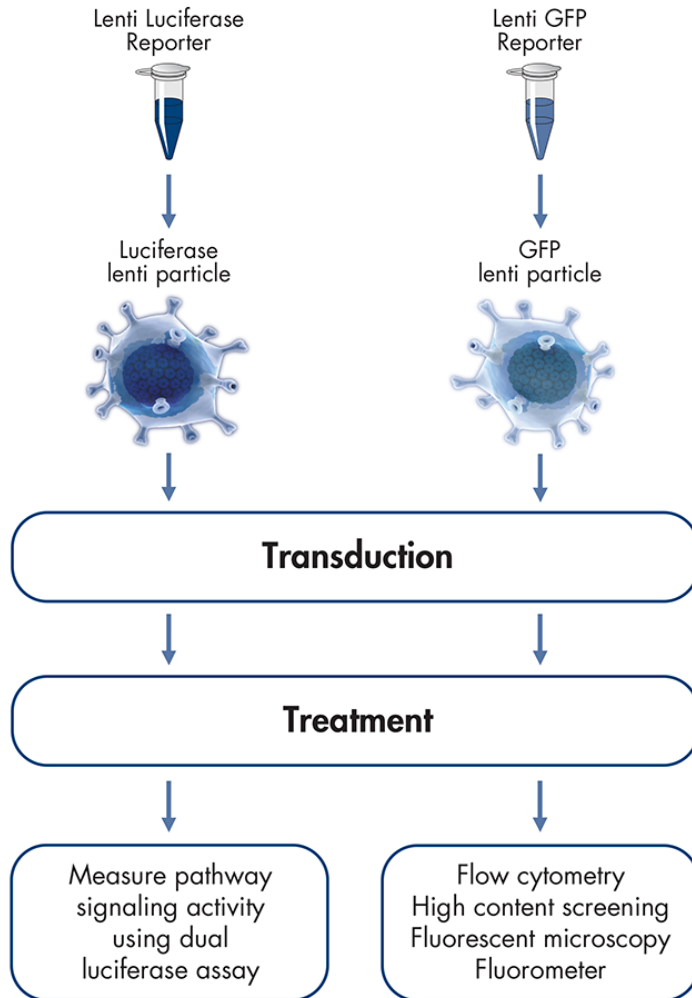
How Signal Reporter Assays (LUC) work



What you need:

- Signal Reporter Assay
- Reliable transfection reagent and protocol (Attractene, HiPerFect)
- Test biological
 - shRNA (SureSilencing shRNA)
 - expression vectors (QIAGENes)
 - protein/peptide
 - small molecule
- Detection instrument and luciferase substrates

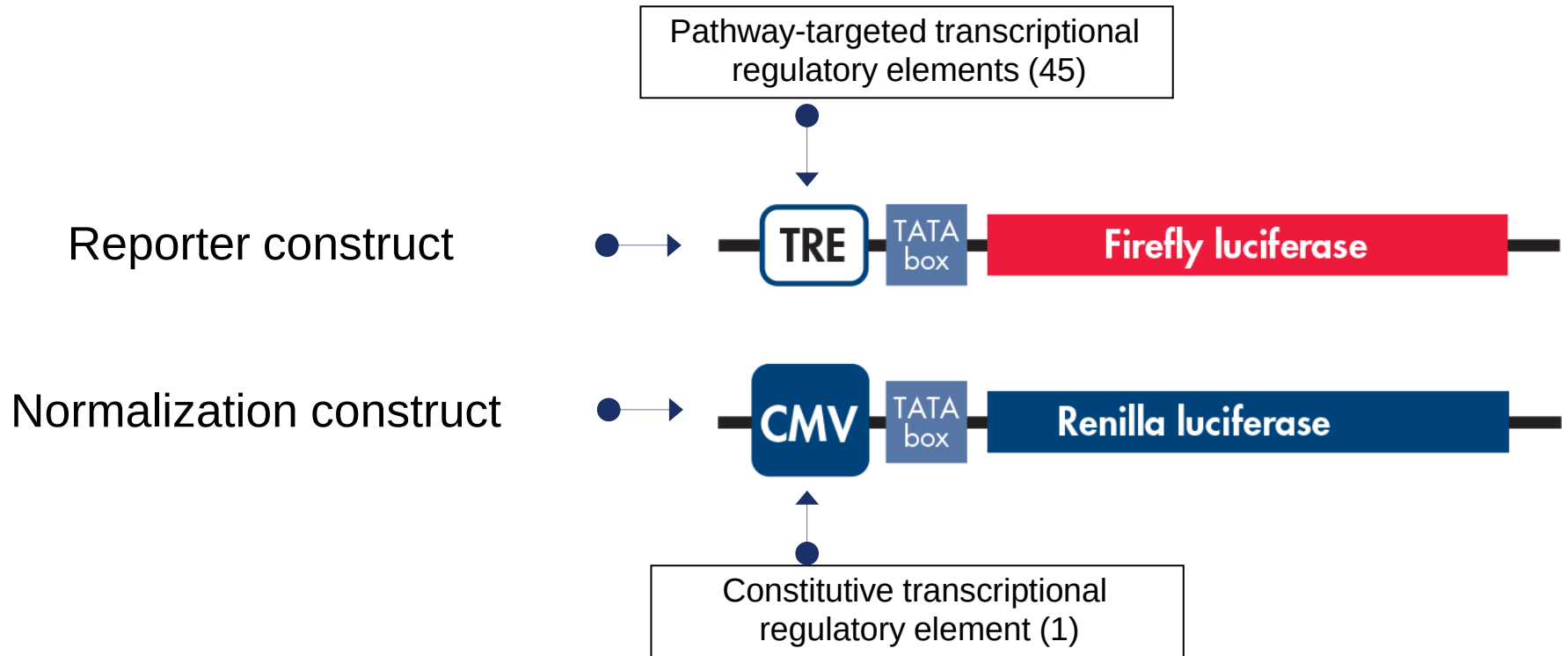
How Signal Lenti Reporter Assays work



What you need:

- Signal Lenti Reporter or 10-Pathway Array
- SureENTRY™ can enhance transduction efficiency in most cell types
- Test biological
 - ☐ siRNA (Flexitube siRNA)
 - ☐ shRNA (SureSilencing shRNA)
 - ☐ expression vectors (Qiagenes)
 - ☐ protein/peptide
 - ☐ small molecule
- Luciferase Assay Kit (Promega) and luminometer or GFP detection system

Key advantages, assay design, dual-luciferase



The Transcriptional Regulatory Elements (TREs) establish the pathway specificity of each reporter!

■ Sources of Variability in Cell-based Assays:

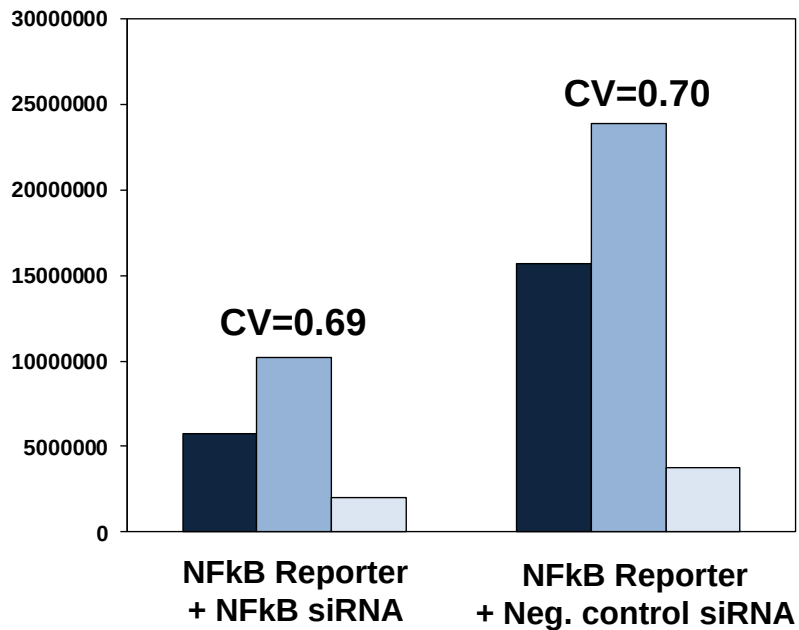
- ☐ Number of cells seeded per well
- ☐ Transfection efficiencies within and across plates
- ☐ Multichannel pipettor inconsistencies
- ☐ “Edge effects” influencing cell culture
- ☐ Viability of cells following transfection/treatment
- ☐ Lysis efficiencies within and across plates
- ☐ Variability inherent to reporter assay

Dual-luciferase assay design corrects for these unwanted sources of variability.

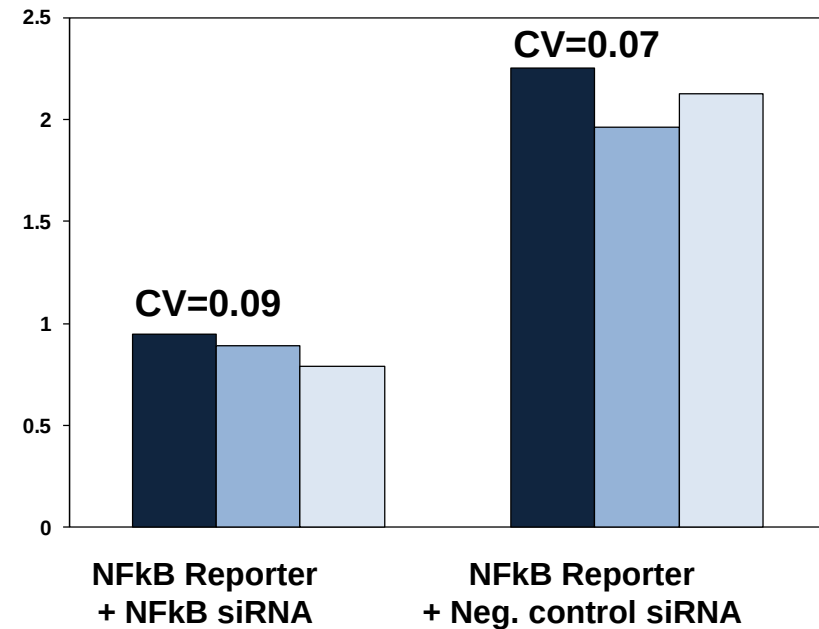
■ Changes due to the designed treatments of cells

- ☐ RNA interference
- ☐ Overexpression
- ☐ Recombinant protein/peptide/growth factor
- ☐ Small molecule

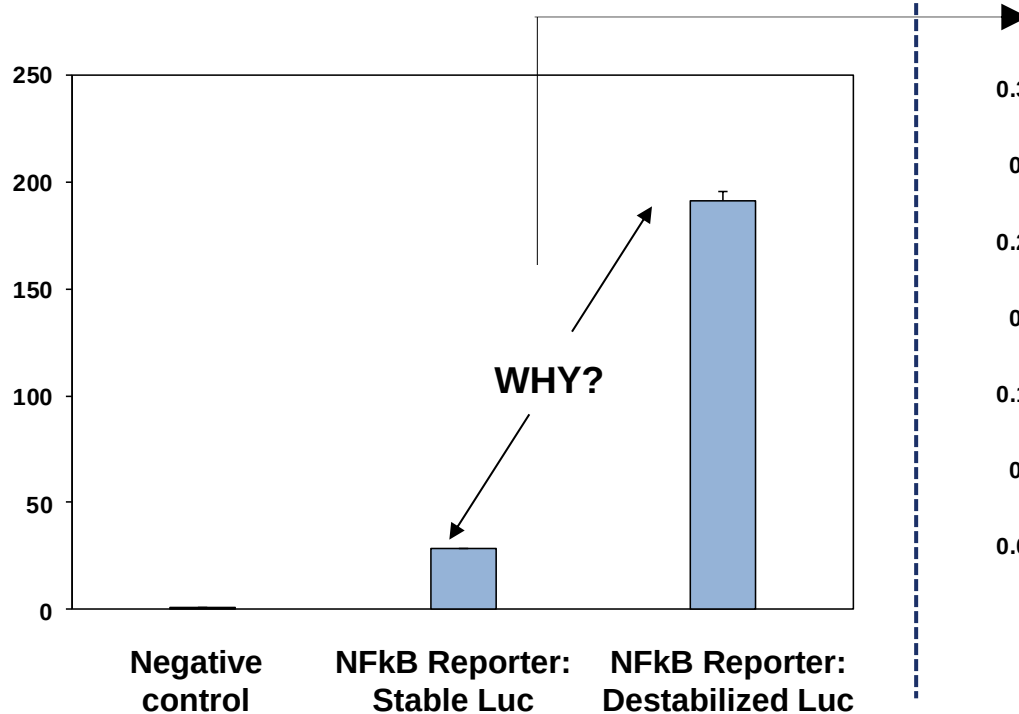
Single-luciferase assays: NFkB Reporter Assay



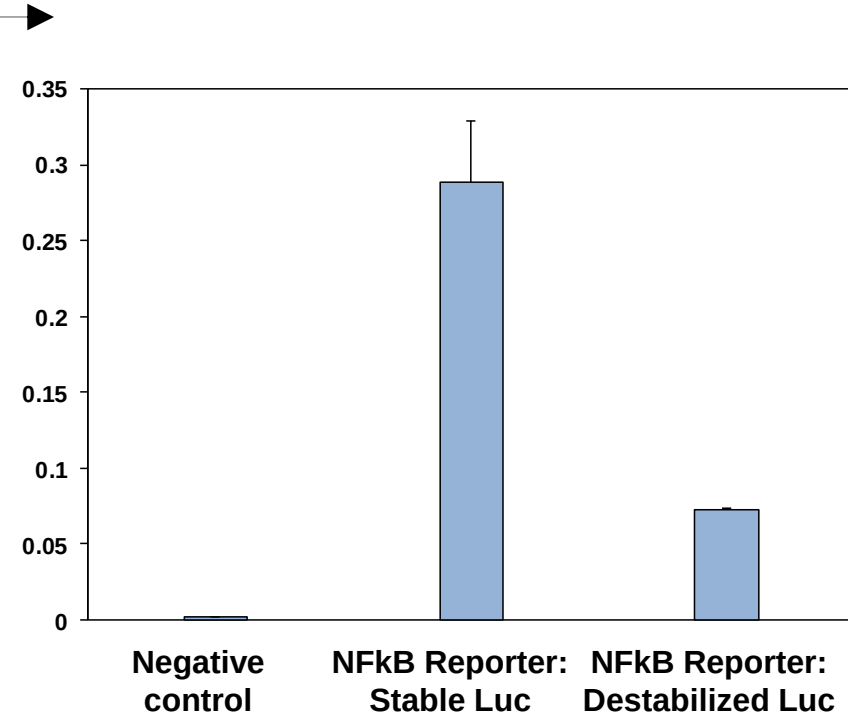
Dual-luciferase assays: NFkB Reporter Assay



Modified luciferase:
NFkB Reporter signal
following $\text{TNF}\alpha$ induction

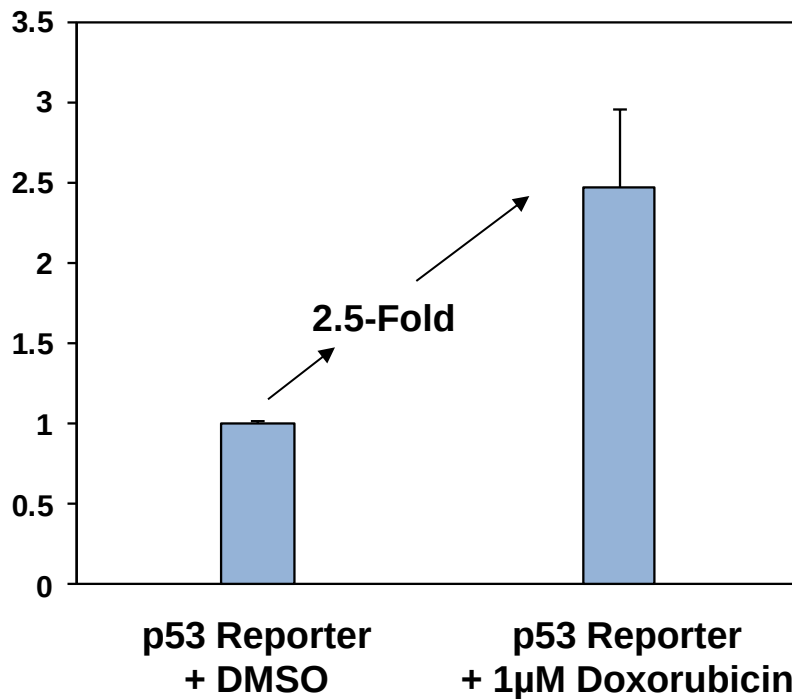


Modified luciferase:
background NFkB Reporter signal
(no treatment post-transfection)

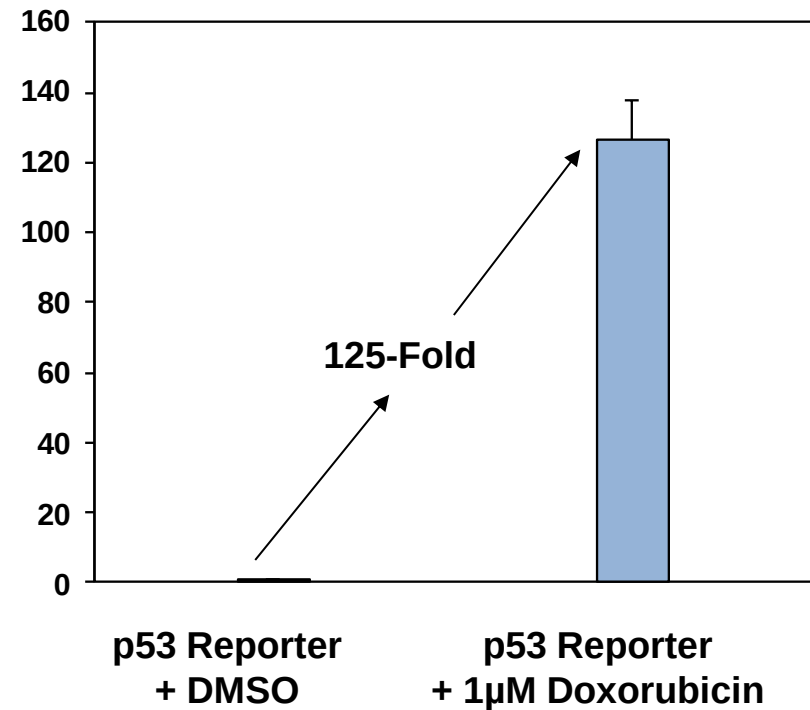


High signal-to-noise ratio
offers higher sensitivity

Dual-luciferase assays utilizing common p53 TRE



Dual-luciferase assays utilizing Signal p53 TRE



Improved sensitivity and biological relevance:

Experimentally optimized response elements :

- Sequence of transcription response element (TRE)
 - Number of TREs
 - Intervening sequence between TRE
- } Maximize specificity and sensitivity

Engineered reporter genes:

- Destabilized firefly luciferase (Maximize signal-to-noise ratio)
- Codon optimization for mammalian expression (Maximize expression)
- Removal of hidden transcription factor binding sites (Reduce background)

Save time, money and labor: use Signal Reporters and Lenti Reporters

DNA-vector GFP – 16 pathways

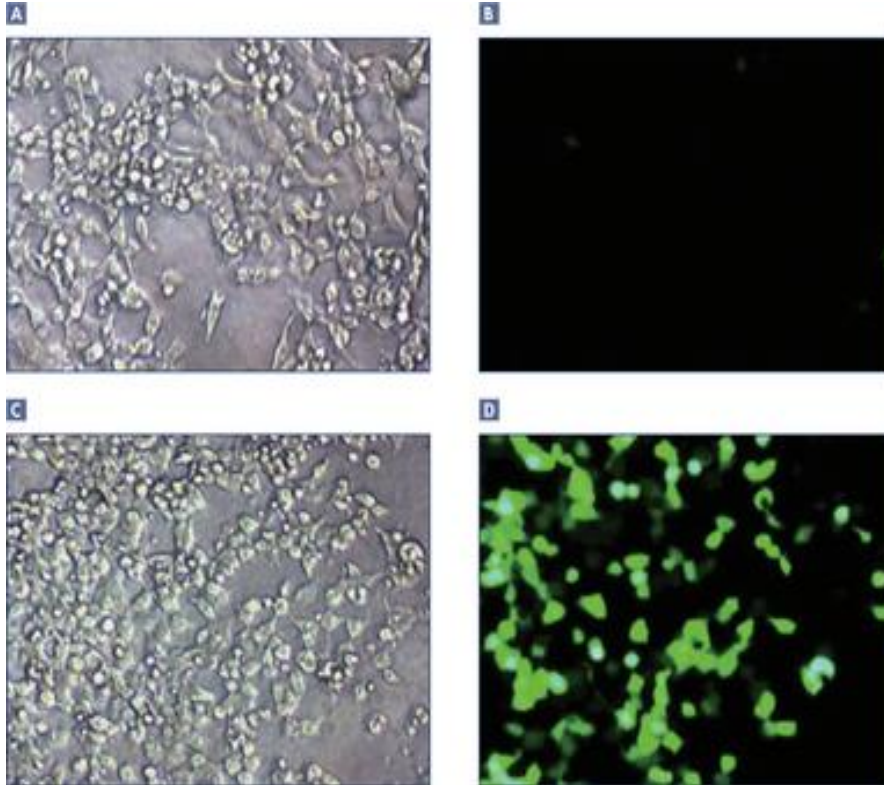
Pathway	Transcription Factor	Pathway	Transcription Factor
Amino Acid Deprivation	ATF4/ATF3/ATF2	MAPK/ERK	Elk-1/SRF
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Glucocorticoid	GR	Progesterone	PR
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HNF4	HNF4	SP1	SP1
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Interferon Gamma	STAT1/STAT1	Wnt	TCF/LEF
KLF4	KLF4	Xenobiotic	AhR
Liver X	LXR		

Lenti-GFP Reporter – 6 pathways

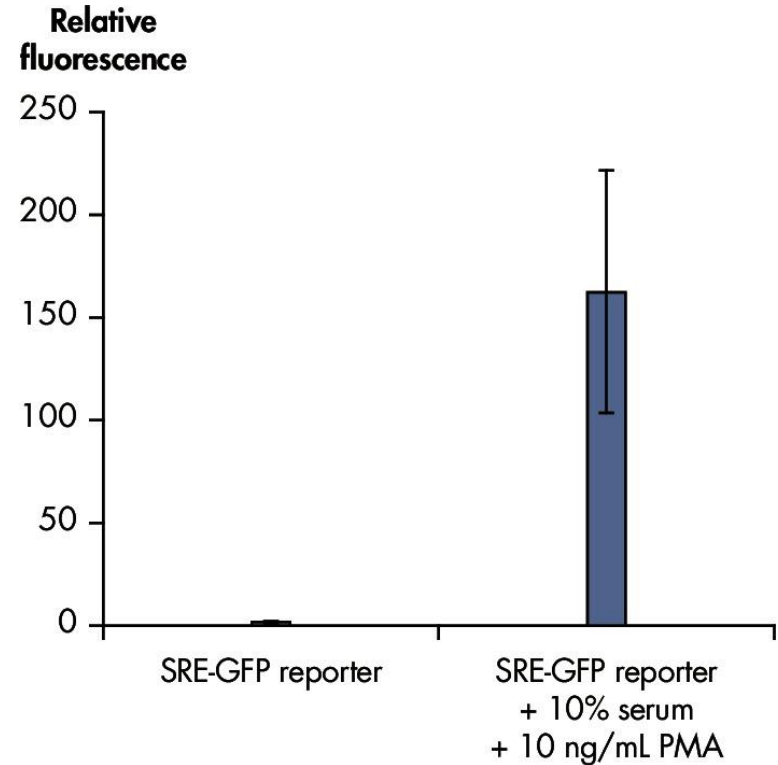
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C/EBP	C/EBP	Nanog	Nanog
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Interferon Gamma	STAT1/STAT1	Wnt	TCF/LEF
KLF4	KLF4	Xenobiotic	AhR
Liver X	LXR		

GFP system application: small molecule studies

Fluorescence microscopy



Fluorometry



Treatment of Cignal SRE (GFP) transfectants with 10% serum and 10 ng/ml PMA activates the MAPK/ERK signaling pathway.

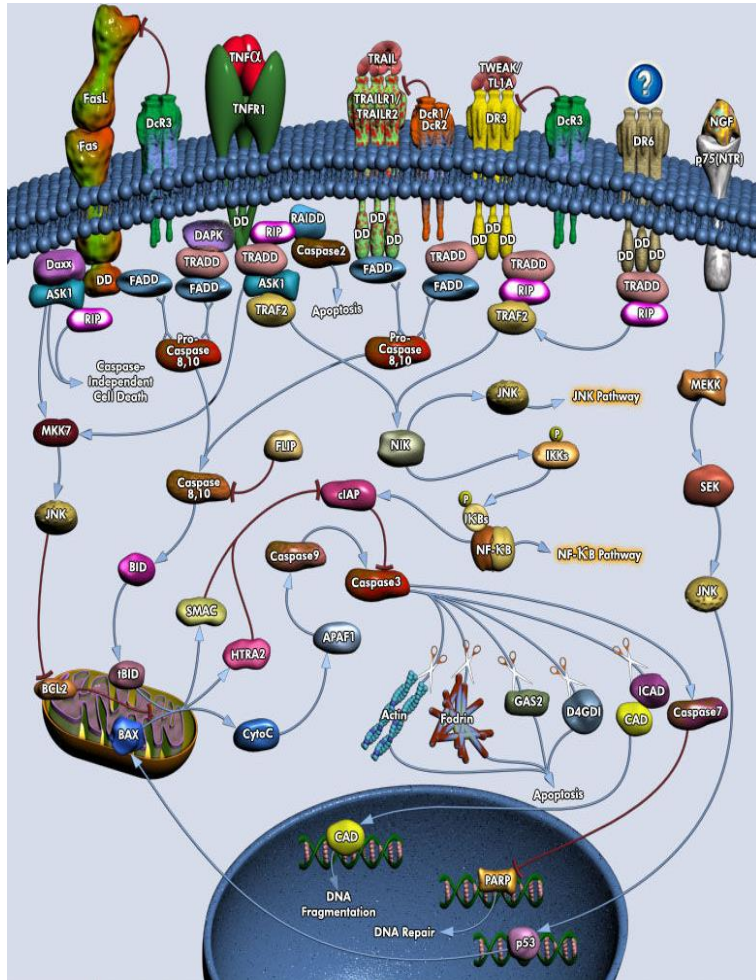
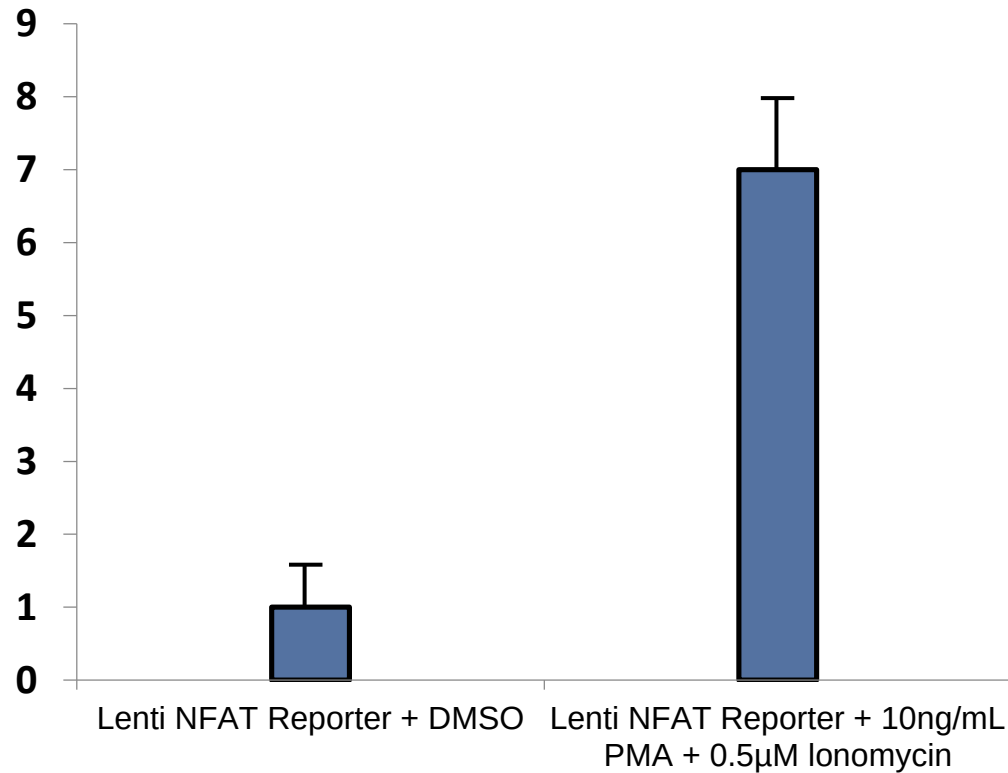


Image reference: [Apoptosis through death receptors. Pathway central, GeneGlobe.](#)

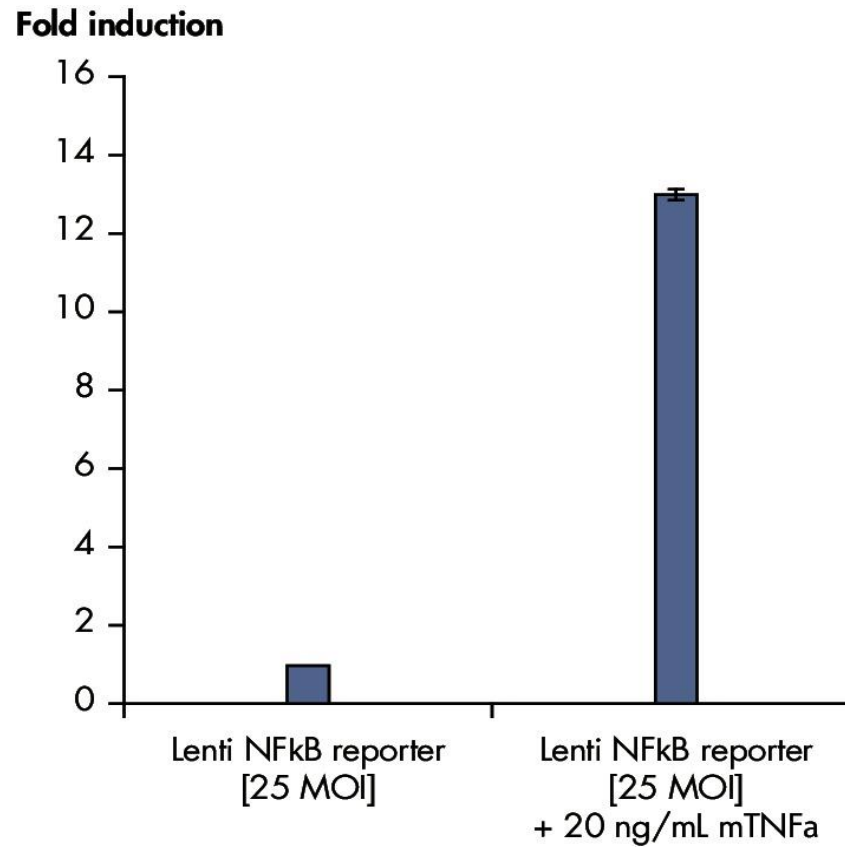
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PKC/ Ca^{++} signaling pathway studies in primary arteriole smooth muscle cells



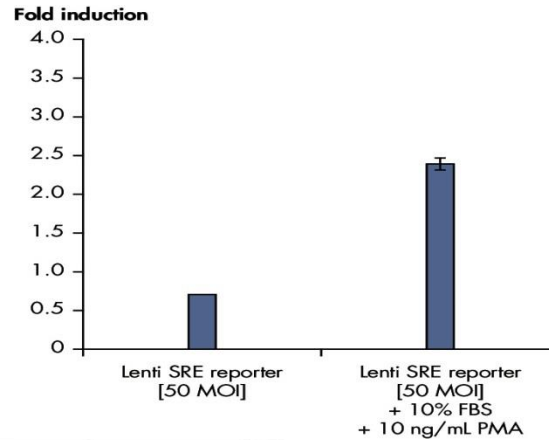
Signal Lenti Reporter system makes this study possible

NFkB signaling pathway studies in D1 Murine T-cell Leukemia cell line

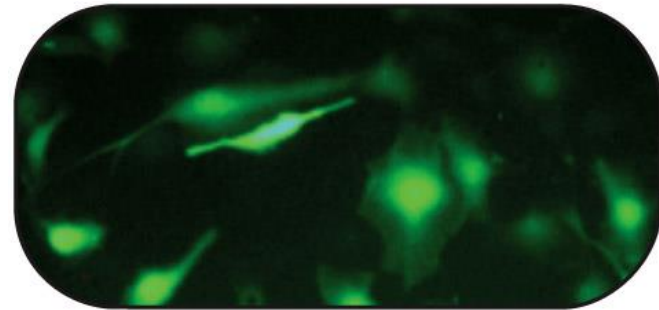


Signal Lenti Reporter system makes this study possible

Signal Lenti SRE Reporter (GFP) measures MAPK/ERK pathway signaling activity in African green monkey CV1 fibroblasts



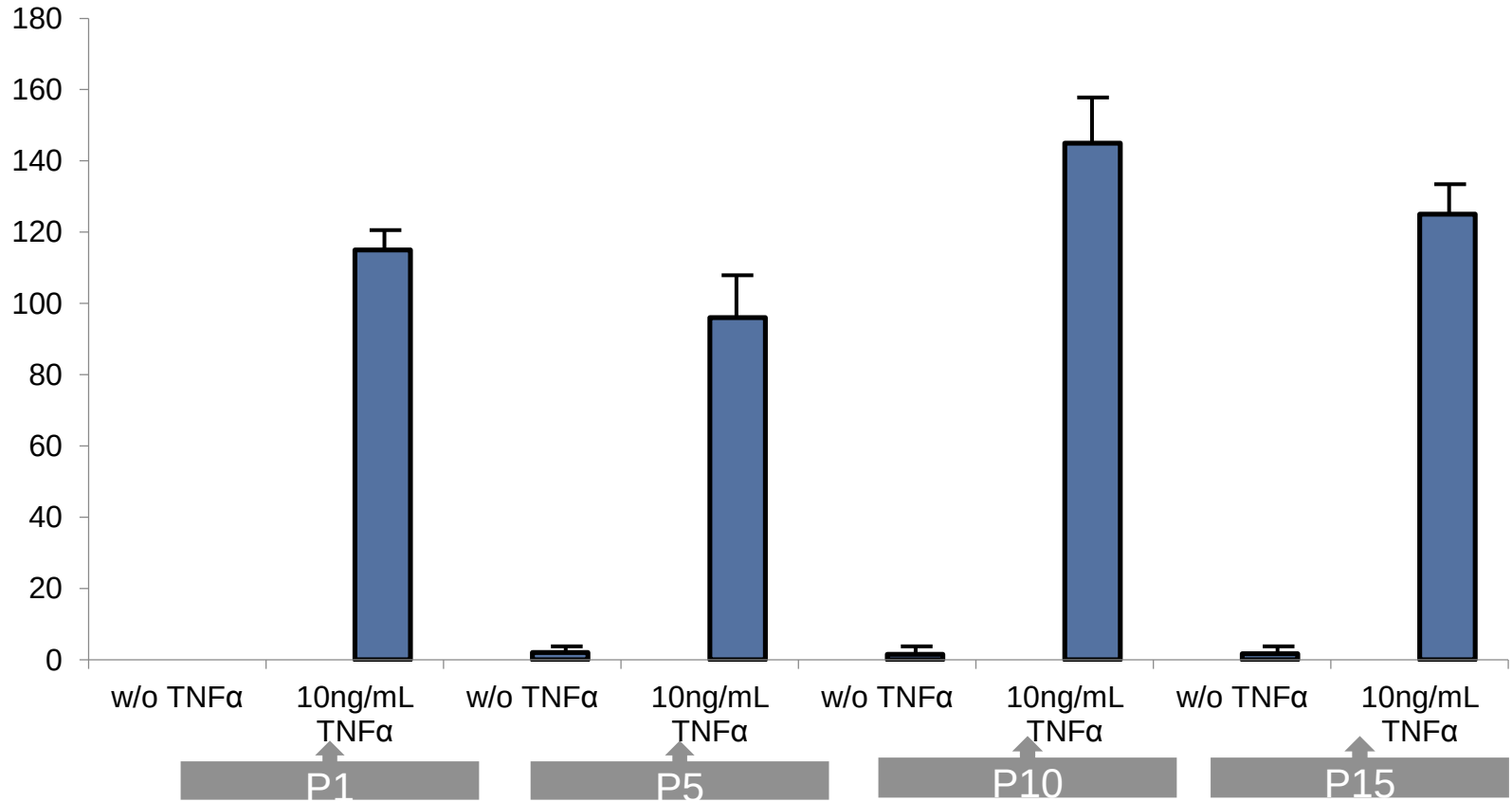
Signal Lenti SRE Reporter (*GFP*) - Untreated



Signal Lenti SRE Reporter (*GFP*) - Treated

Signal Lenti Reporter system makes this study possible

Generation of an NFkB signaling pathway stable sensor cell line



Signal Lenti Reporters enable rapid pathway sensor cell line generation

Functional genomics

- What's the phenotype of my gene?
- Does my gene of interest regulate signaling pathway "X"?

Functional proteomics

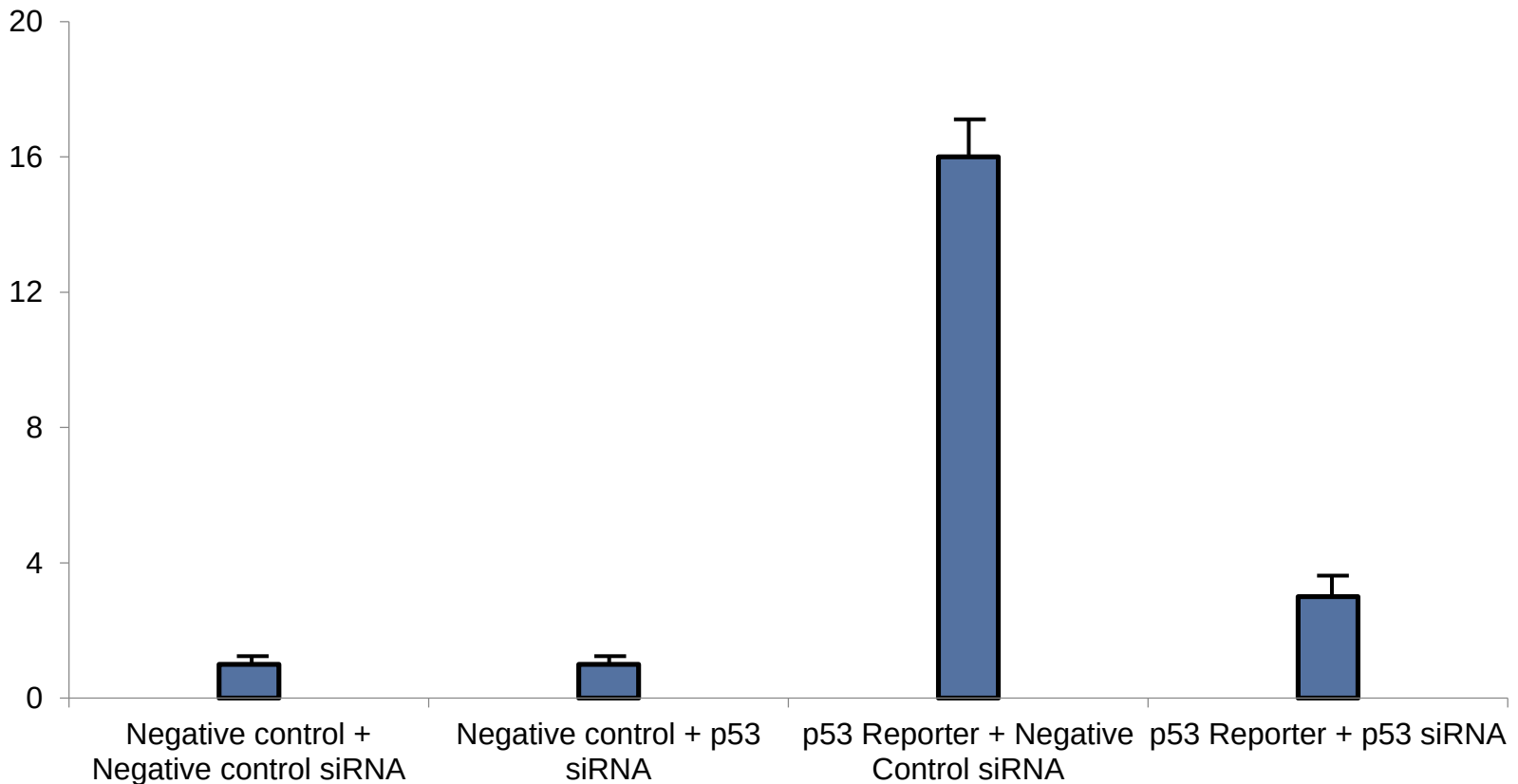
- What's the phenotype of my protein/peptide?
- Does my protein, peptide, or growth factor of interest regulate signaling pathway "X"?

Drug screening

- What is the mechanism of action of my small molecule drug candidates?
- Do my drug candidates of interest regulate signaling pathway "X"?

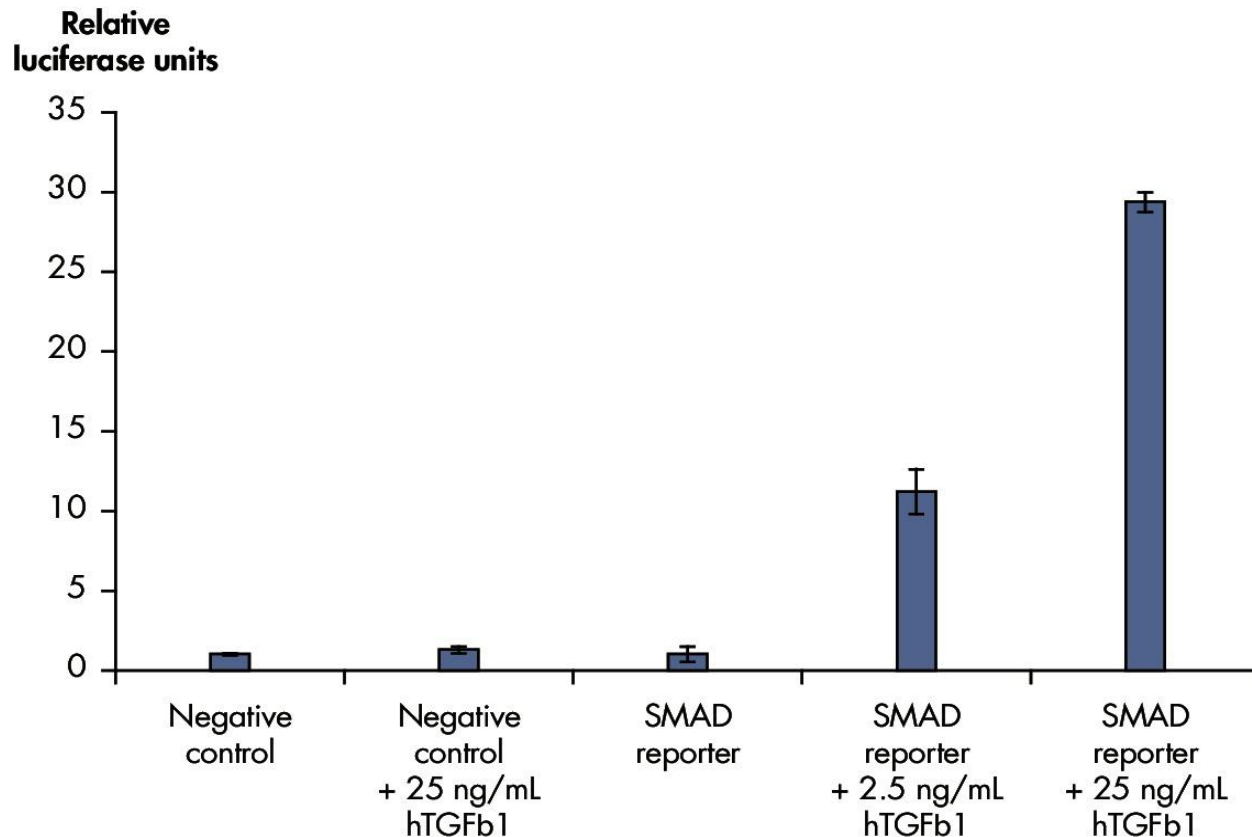
Functional genomics

- What's the phenotype of my gene?
- Does my gene of interest regulate signaling pathway “X”?



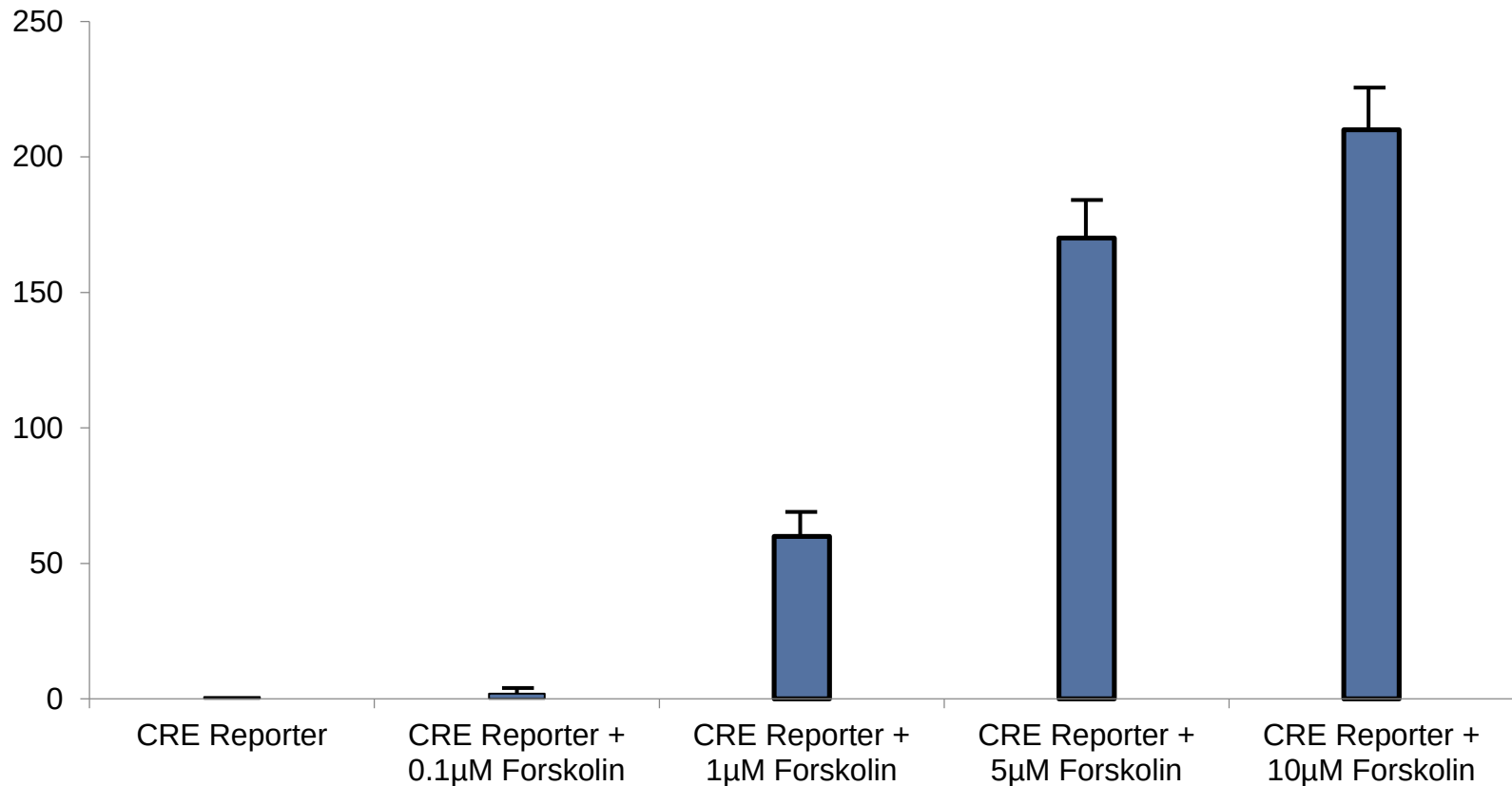
Functional proteomics

- What's the phenotype of my protein/peptide?
- Does my protein, peptide, or growth factor of interest regulate signaling pathway "X"?



Drug screening

- What is the mechanism of action of my small molecule drug candidates?
- Do my drug candidates of interest regulate signaling pathway “X”?



Situation:

I have a biological molecule that knocks down p53 expression

Questions:

- What is impact of p53 gene expression knock down on the p53 signaling pathway?
- What is the impact of p53 knock down on other cancer-relevant signaling pathways?

Wnt signaling

p53 signaling

NFkB signaling

E2F signaling

MAPK/JNK
signaling

Hypoxia
signaling

TGFβ signaling

MAPK/ERK
signaling

Notch signaling

Myc signaling

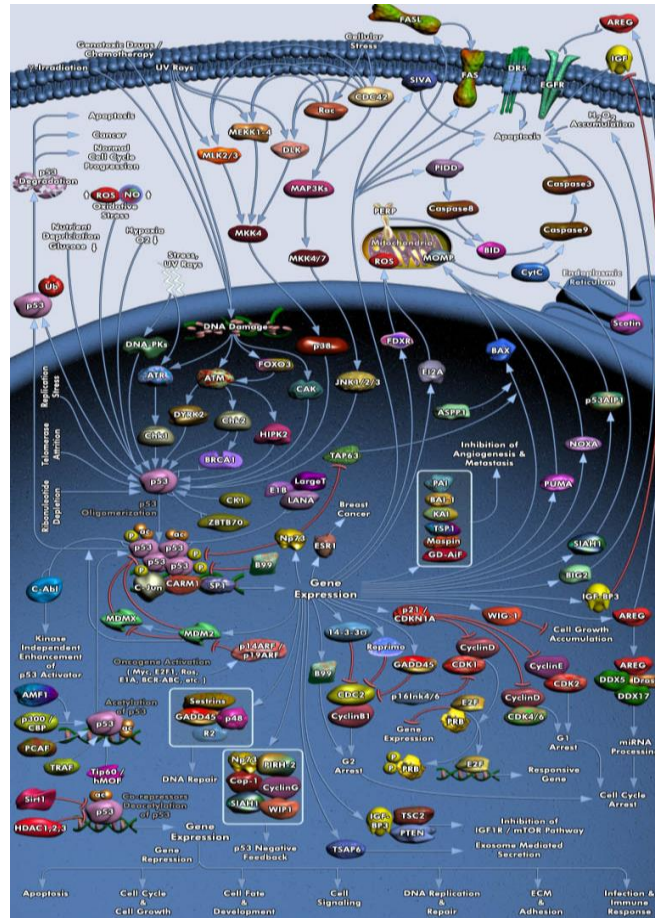


Image reference: [p53signaling. Pathway central, GeneGlobe.](#)

Approach:

Carry out a p53 RNA interference experiment with multiple cancer-relevant Signal Reporter Assays

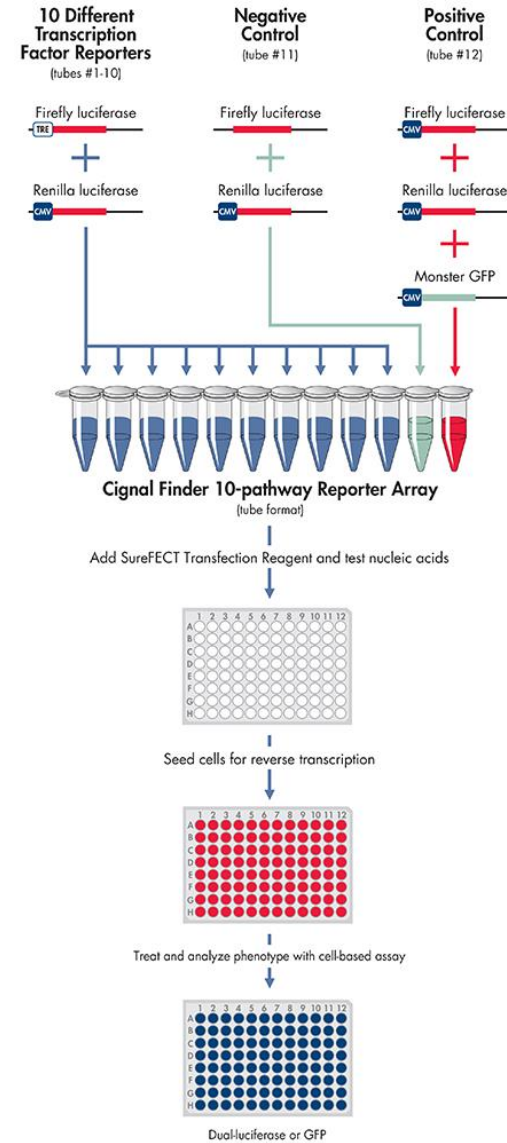
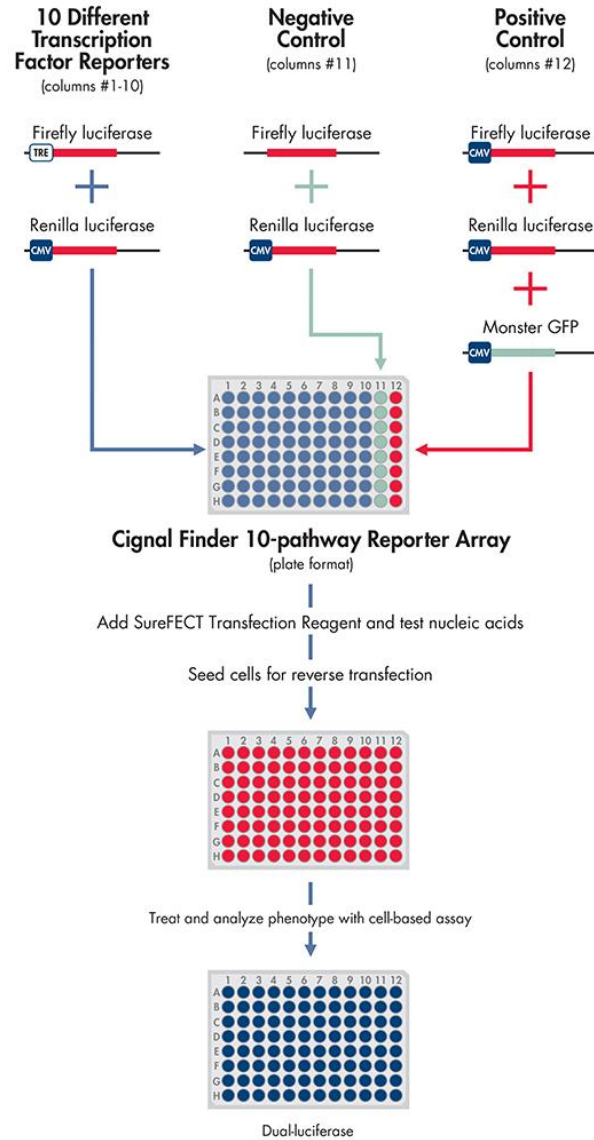
Pathway

Transcription Factor

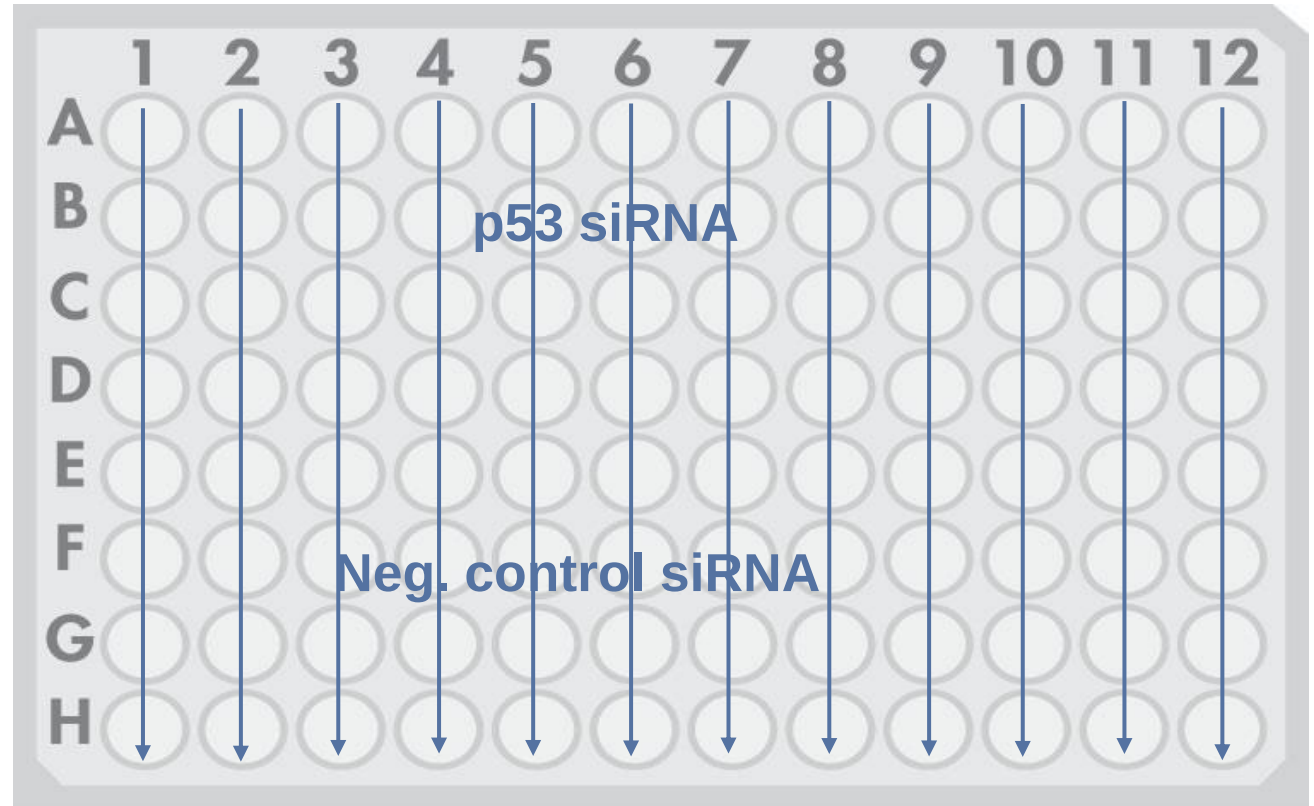
Cell Cycle
DNA Damage
Hypoxia
MAPK/ERK
MAPK/JNK
c-Myc
NFkB
Notch
TGFβ
Wnt

E2F/DP1
p53
HIF
Elk-1/SRF
AP1
Myc/Max
NFkB
RBP-Jk
SMAD2/SMAD3/SMAD4
TCF/LEF

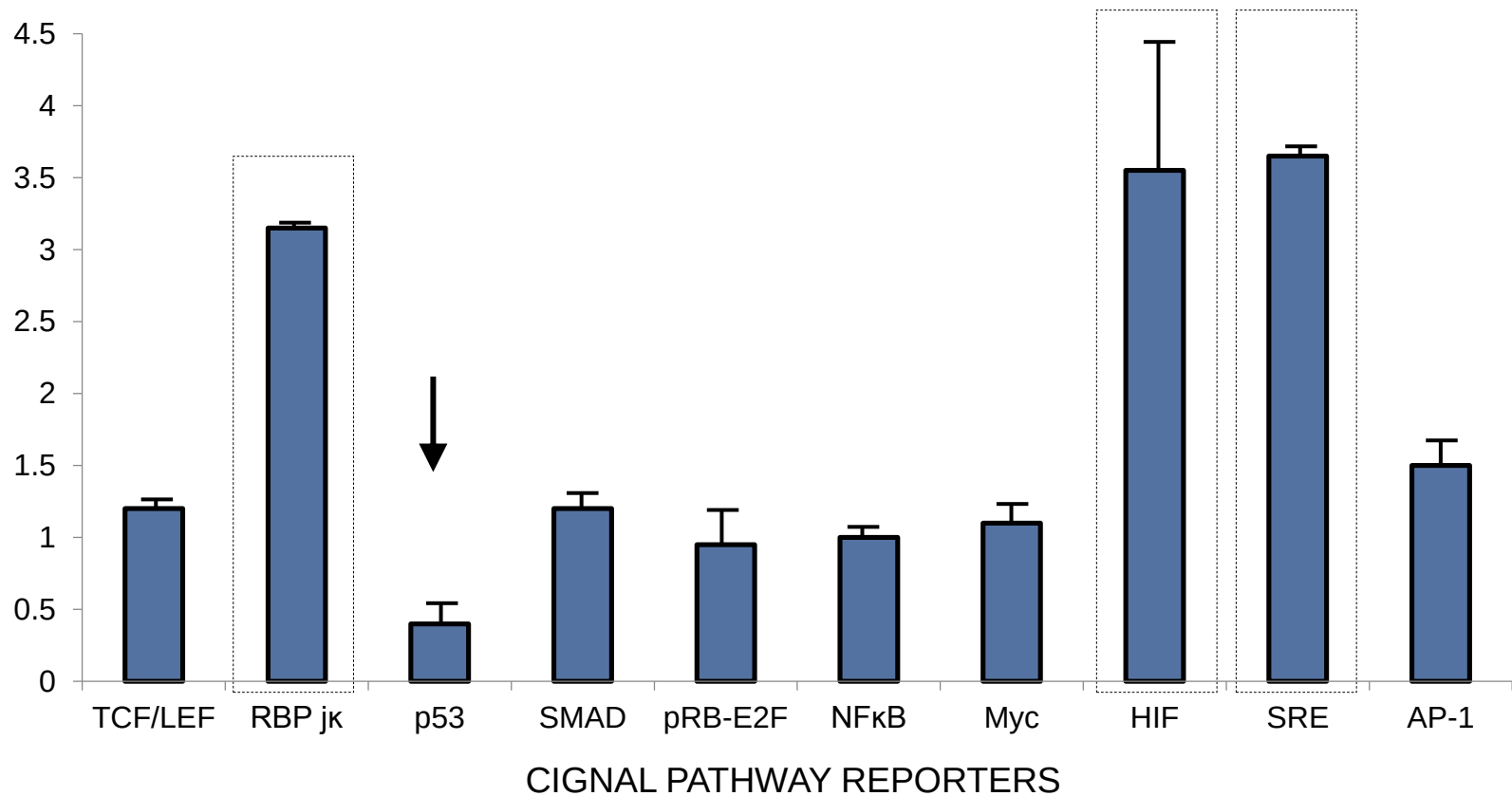
Signal Finder Array: how does it work?



1. p53 Signal Reporter
2. RBP-Jk Signal Reporter
3. TCF/LEF Signal Reporter
4. E2F Signal Reporter
5. AP1 Signal Reporter
6. SRE Signal Reporter
7. SMAD Signal Reporter
8. NFkB Signal Reporter
9. Myc Signal Reporter
10. HIF Signal Reporter
11. Signal Negative control
12. Signal Positive control



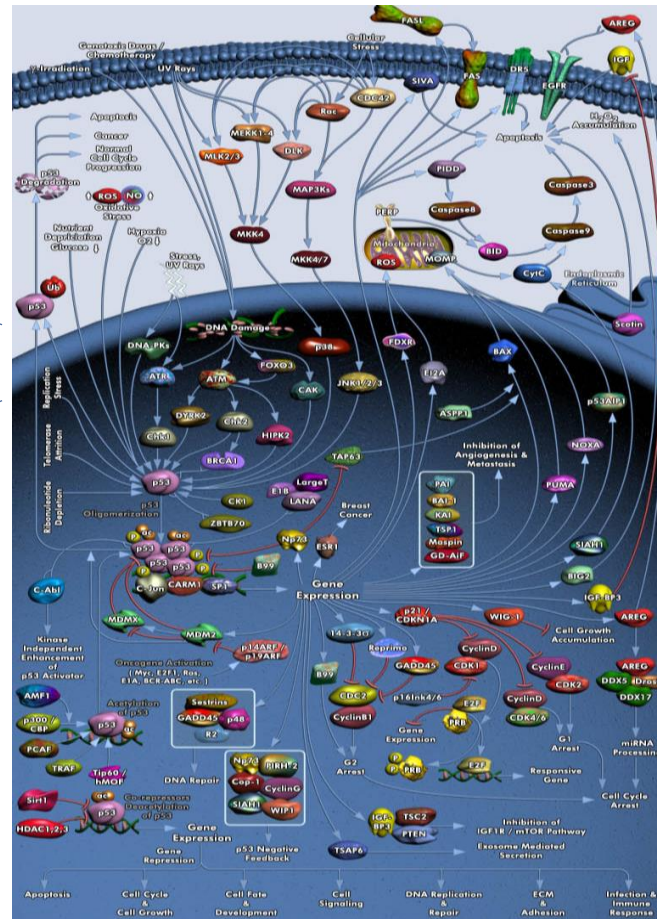
Impact of p53 siRNA treatment



p53 signaling

Hypoxia
signaling

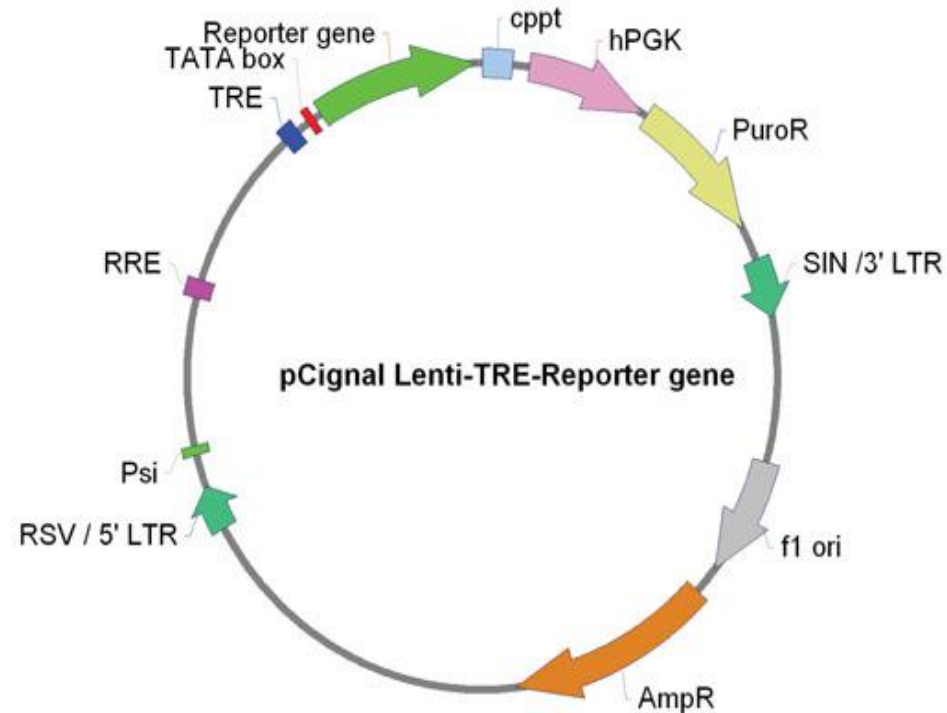
MAPK/ERK
signaling



Notch signaling

Image reference: [p53signaling](#). Pathway central, GeneGlobe.

- VSV-G pseudotyped lentiviral particles
- Deletion in U3 portion of 3'LTR results in **self-inactivation (SIN)**, following transduction of target cell
- No structural or replication genes are expressed in transduced cells, making Signal lentiviral vectors **replication-defective**
- No virulence genes (vpr, vif, vpu, nef) are present in the Signal Lenti Reporters
- Experiments should be performed under **Biosafety Level 2 (BSL-2)** conditions



See our Signal Lenti Reporter web pages for useful links

Avoid freeze thaws

- Result in activity loss

MOI dilution series

- Recommended range 5–50

Transduction reagent

- Sure ENTRY usually in the range of 4–8 $\mu\text{g/ml}$

MOIs >50 should be split

- Sequential 4-hour transductions

Kill curve for Puromycin

- Don't guess 500–10,000 ng/ml

MOI for stables

- Perform a transient study to find appropriate MOI

Freeze cells down

- Renewable reagent

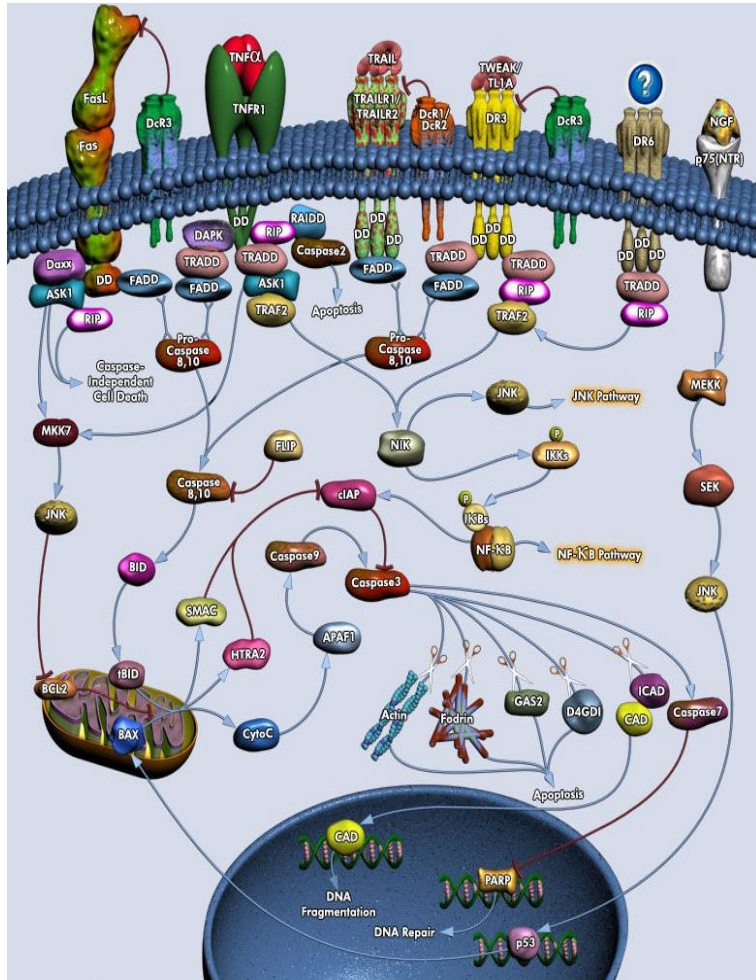


Image reference: [Apoptosis through death receptors. Pathway central, GeneGlobe.](#)

- 1 [Taking on challenges with Signal Reporter Assays](#)
- 2 [How Signal Reporter and Lenti Assays work](#)
- 3 [How you can use Signal Reporter Assays in your research](#)
- 4 [Summary](#)

Breadth of pathways and arrays

- 35 pathway-focused reporters
- 2 application-focused Signal Finder Lenti 10-Pathway Arrays

Performance

- Exceptional sensitivity, specificity, signal-to-noise ratio and reproducibility, using either Luciferase or GFP reporter formats

High-efficiency lentiviral delivery system

- Study cell signaling in primary cells, stem cells and difficult to transfect cell lines
- Rapidly generate pathway sensor cell lines

Final benefit to researchers:

- Ready-to-use, validated reporters
- Don't have to produce or amplify lentivirus in your laboratory
- Quick generation of reporter cell lines

Breadth of pathways and research application

- 45 pathway-focused reporter assays
- 6 application-specific Signal Finder 10-Pathway Arrays
- 1 Signal 45-Pathway Reporter Array

Performance

- Exceptional sensitivity, specificity, signal-to-noise ratio and reproducibility

Convenience

- Ready-to-use, validated, preformatted transient assays

Dual Modalities

- Dual-luciferase quantitative end-point assays
- GFP dynamic live cell assays with single cell resolution

Questions?

Contact technical support 9 AM – 6 PM EST

Telephone: 800-362-7737

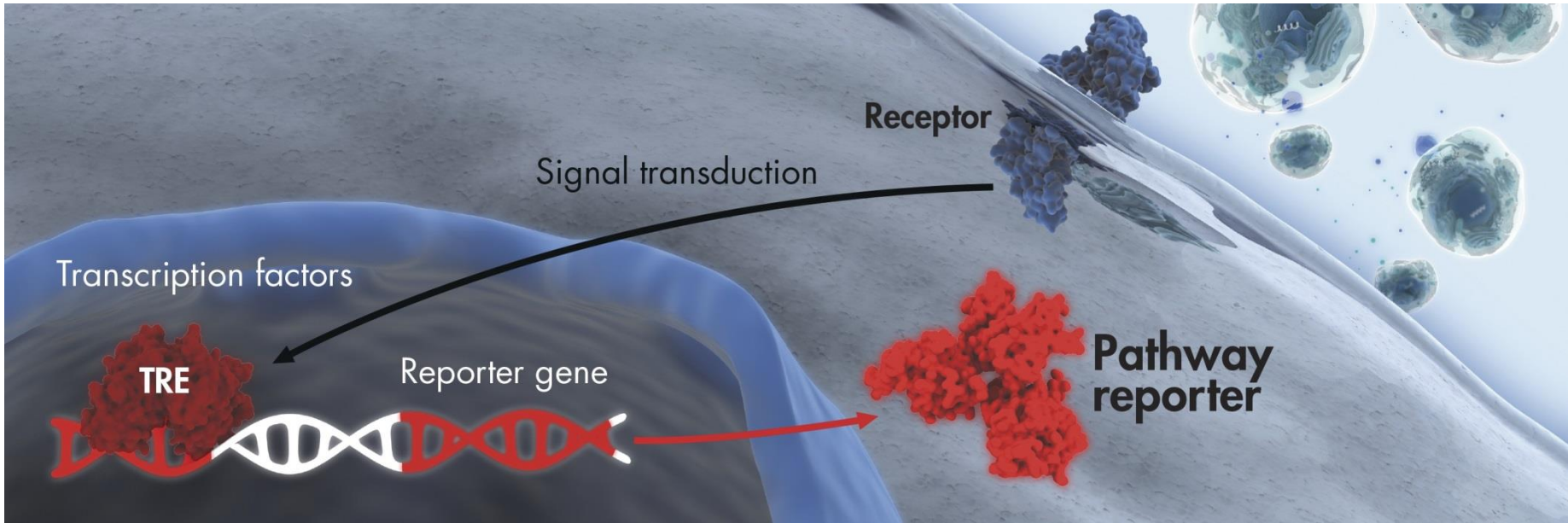
Or

Contact Anisha Kharkia

Global Product Manager

Email: Anisha.Kharkia@qiagen.com

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Thank you