Cignal™ Reporter Assays

Simple and Robust Signal Transduction Assays

Cignal Reporter Assays
Simplifying Cell-Based Gene & Pathway Function Studies

Brian McNally, Ph.D.
Associate Product Manager
Topics to be Covered

- **Who is SABiosciences?**

- **Utility of the Cignal Reporter Assays**
  - Challenges Associated with Cell-Based Assays
  - Solutions Cignal Reporter Assays Provide

- **How Cignal Reporter Assays Work**
  - Cignal Pathway Reporters Product Family
  - Dual-Luciferase Assays
  - Luciferase Engineered for High Performance
  - Optimized Transcriptional Regulatory Elements (TREs)

- **How YOU Can Use Cignal Reporter Assays**
  - Performance and applications data.
Who IS SABiosciences?

- Located in Frederick, Maryland, USA (near to NIH Bethesda campus)
- Founded 10 years ago by scientists working at the NIH
- **Core Values:** Innovation, Focus, Systematic Design
- **Goal:** Transform cutting-edge biological information and technologies into easy to use, accessible, and innovative systems biology research tools.
- **Products:** Gene expression analysis, protein function analysis and cell based assays
# Systems Biology @ SABiosciences

<table>
<thead>
<tr>
<th>Research</th>
<th>Technologies</th>
<th>SABiosciences Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>Sequencing, SNP Detection, ChIP, Methylation</td>
<td>ChIP-qPCR Assays, Methyl Profiler</td>
</tr>
<tr>
<td>Genomics &amp; Regulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNA</td>
<td>Discovery Screening, Confirmation</td>
<td>Gene Expression Service Core, RT² PCR Arrays, RT² miRNA Arrays and Assays, RT² qPCR Assays</td>
</tr>
<tr>
<td>Gene Expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>Detection, Knockdown, Reporter System, Phenotypes</td>
<td>ELISA, Array Kits, siRNA Arrays / shRNA Plasmids, Cignal Reporter System, CASE Kits</td>
</tr>
<tr>
<td>Gene Function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein Function</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Focus on Your Pathway™*
Topics to be Covered

• Who is SABiosciences?

• Utility of the Cignal Reporter Assays
  • Challenges Associated with Cell-Based Assays
  • Solutions Cignal Reporter Assays Provide

• How Cignal Reporter Assays Work
  • Cignal Pathway Reporters Product Family
  • Dual-Luciferase Assays
  • Luciferase Engineered for High Performance
  • Optimized Transcriptional Regulatory Elements (TREs)

• How YOU Can Use Cignal Reporter Assays
  • Performance and applications data.
Cignal Applications

• Monitoring signaling pathway activation
  Is my pathway of interest regulated by biological molecule “X”?  

• Studying the biological response of a drug candidate or chemical compound
  Does my compound of interest regulate pathway “X”?

• Phenotypic analysis of gene knockdown or gene over-expression
  Does my gene of interest regulate pathway “X”?  

• Mode of action of protein, peptide and ligands
  Does my protein/peptide/ligand of interest regulate pathway “X”?
Cell-based Assay Research Challenges

- **Variability within experiments**
  Well-to-well and plate-to-plate reproducibility are notoriously challenging with cell-based assays.

- **Variability between experiments**
  Reliable results often depend upon the “status” of the cells, and who in the lab is conducting the experiments.

- **Assay Performance**
  Sensitivity, specificity, signal to noise ratio, and simplicity are of utmost importance.
What Cignal Reporter Assays Provide

- Functionally validated dual-reporter formulation
  Minimizes variability, increasing the biological relevance of each experiment

- Ready-to-use reporters and controls, coupled with a transient reporter system
  Enables simple and rapid analysis of the regulation of 29 signal transduction pathways

- Combination of custom-engineered transcriptional regulatory elements (TREs) and destabilized, codon-optimized firefly luciferase
  Increases the signal to noise ratio and maximizes assay sensitivity and specificity
Topics to be Covered

- **Who is SABiosciences?**
- **Utility of the Cignal Reporter Assays**
  - Challenges Associated with Cell-Based Assays
  - Solutions Cignal Reporter Assays Provide

- **How Cignal Reporter Assays Work**
  - Cignal Pathway Reporters Product Family
  - Dual-Luciferase Assays
  - Luciferase Engineered for High Performance
  - Optimized Transcriptional Regulatory Elements (TREs)

- **How YOU Can Use Cignal Reporter Assays**
  - Performance and applications data.
Cignal Pathway Reporter System Products

- Easy to transfect cell lines
  - Endpoint Assays
    - Cignal Dual-Luciferase Reporter Assays
      - Single Pathway Assays
      - 10-Pathway Arrays
  - Dynamic Live Cell Assays
    - Cignal GFP Reporter Assays
      - Single Pathway Assays
  - Endpoint Assays
    - Dynamic Live Cell Assays

- Primary cells, stem cells, and difficult to transfect cell lines
  - Endpoint Assays
  - Dynamic Live Cell Assays
  - Cignal Lenti Luciferase Reporters
    - Single Pathway Assays
    - 10-Pathway Arrays
  - Cignal Lenti GFP Reporters
    - Single Pathway Assays
## Cignal Reporter Assays

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Transcription Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino Acid Deprivation</td>
<td>ATF4/ATF3/ATF2</td>
</tr>
<tr>
<td>Androgen</td>
<td>Androgen Receptor</td>
</tr>
<tr>
<td>Antioxidant Response</td>
<td>Nrf2 &amp; Nrf1</td>
</tr>
<tr>
<td>ATF6</td>
<td>ATF6</td>
</tr>
<tr>
<td>C/EBP</td>
<td>C/EBP</td>
</tr>
<tr>
<td>cAMP/PKA</td>
<td>CREB</td>
</tr>
<tr>
<td>Cell Cycle</td>
<td>E2F/DP1</td>
</tr>
<tr>
<td>DNA Damage</td>
<td>p53</td>
</tr>
<tr>
<td>EGR1</td>
<td>EGR1</td>
</tr>
<tr>
<td>ER Stress</td>
<td>CBF/NF-Y/YY1</td>
</tr>
<tr>
<td>Estrogen</td>
<td>ER</td>
</tr>
<tr>
<td>GATA</td>
<td>GATA</td>
</tr>
<tr>
<td>Glucocorticoid</td>
<td>GR</td>
</tr>
<tr>
<td>Heat Shock Response</td>
<td>HSF</td>
</tr>
<tr>
<td>Heavy Metal Stress</td>
<td>MTF1</td>
</tr>
<tr>
<td>Hedgehog</td>
<td>GLI</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>HIF-1</td>
</tr>
<tr>
<td>Type I Interferon</td>
<td>STAT1/STAT2</td>
</tr>
<tr>
<td>Interferon Gamma</td>
<td>STAT1/STAT1</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathway</td>
<td>Transcription Factor</td>
</tr>
<tr>
<td>MAPK/ERK</td>
<td>Elk-1/SRF</td>
</tr>
<tr>
<td>MAPK/JNK</td>
<td>AP-1</td>
</tr>
<tr>
<td>MEF2</td>
<td>MEF2</td>
</tr>
<tr>
<td>c-myc</td>
<td>Myc/Max</td>
</tr>
<tr>
<td>NF κ B</td>
<td>NF κ B</td>
</tr>
<tr>
<td>Notch</td>
<td>RBP-Jk</td>
</tr>
<tr>
<td>Oct4</td>
<td>Oct4</td>
</tr>
<tr>
<td>PI3K/akt</td>
<td>FOXO</td>
</tr>
<tr>
<td>PKC/Ca++</td>
<td>NFAT</td>
</tr>
<tr>
<td>PPAR</td>
<td>PPAR</td>
</tr>
<tr>
<td>Retinoic Acid</td>
<td>RAR</td>
</tr>
<tr>
<td>SP1</td>
<td>SP1</td>
</tr>
<tr>
<td>STAT3</td>
<td>STAT3</td>
</tr>
<tr>
<td>TGF β</td>
<td>SMAD2/3/4</td>
</tr>
<tr>
<td>VDRE</td>
<td>Vitamin D Receptor</td>
</tr>
<tr>
<td>Wnt</td>
<td>TCF/LEF</td>
</tr>
</tbody>
</table>

**Focus on Your Pathway™**
REPORTER DESIGN: Dual-Luciferase Assays

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

Reporter Construct

\[
\text{TRE} \quad \text{TATA box} \quad \text{Firefly luciferase}
\]

Normalization Construct

\[
\text{CMV} \quad \text{TATA box} \quad \text{Renilla luciferase}
\]

Pathway-targeted transcriptional regulatory elements (35)

Constitutive transcriptional regulatory element (1)
Using Cignal Reporters: What You Need

- Cignal Reporter Assay
- Reliable transfection reagent and protocol (SureFECT)
- Test Biological
  - siRNA (SureSilencing siRNA Arrays)
  - shRNA (SureSilencing shRNA)
  - expression vectors
  - protein/peptide
  - small molecule
- Detection Instrument and Luciferase Substrates
The Transcriptional Regulatory Elements (TREs) are what establish the pathway specificity of each reporter!
Dual Luciferase Assays: Why Is It Important?

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
- Changes due to the designed treatments of cells
  - RNA interference
  - Overexpression
  - Recombinant protein/peptide/growth factor
  - Small molecule

Dual-Luciferase Assay design corrects for these unwanted sources of variability.
Dual Luciferase Format Minimizes Variability

Dual-Luciferase Assays: NFkB Reporter Assay

- CV=0.07
- CV=0.09

Single-Luciferase Assays: NFkB Reporter Assay

- CV=0.69
- CV=0.70
REPORTER DESIGN: Dual-Luciferase Assays

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

**Reporter Construct**

- **TRE**
- **TATA box**
- **Firefly luciferase**

**Normalization Construct**

- **CMV**
- **TATA box**
- **Renilla luciferase**

Mammalian codon-optimized
AND
Destabilized firefly luciferase
Engineered Luciferase and the Signal:Noise Ratio

Modified Luciferase: NFκB Reporter Signal Following TNFα Induction

Modified Luciferase: Background NFκB Reporter Signal
(no treatment post-transfection)

WHY?

- Negative Control
- NFκB Reporter: Stable Luc
- NFκB Reporter: Destabilized Luc

Fold Induction

Relative Luciferase Units

Negative Control
NFκB Reporter: Stable Luc
NFκB Reporter: Destabilized Luc

Focus on Your Pathway™
The Transcriptional Regulatory Elements (TREs) are what establish the pathway specificity of each reporter!

**Reporter Construct**
- **TRE**
- **TATA box**
- **Firefly luciferase**

**Normalization Construct**
- **CMV**
- **TATA box**
- **Renilla luciferase**
Cignal TRE Maximize Luciferase Signal

**Dual-Luciferase Assays Utilizing Cignal p53 TRE**

![Graph showing 125-Fold increase in relative luciferase units from p53 Reporter + DMSO to p53 Reporter + 1µM Doxorubicin.]

**Dual-Luciferase Assays Utilizing Common p53 TRE**

![Graph showing 2.5-Fold increase in relative luciferase units from p53 Reporter + DMSO to p53 Reporter + 1µM Doxorubicin.]

Focus on Your Pathway™
Cignal Reporter Assays: How It Works

**How Cignal Reporter (Luc) Assays Work**

- **Transfection**
- **Treatment**
- Measure Pathway Signaling Activity Using Dual Luciferase Assay
- Assess Transfection Efficiency

**How Cignal Reporter (GFP) Assays Work**

- **Transfection**
- **Treatment**
- Flow Cytometry High Content Screening Fluorescent Microscopy Fluorometer
- Assess Transfection Efficiency
# Cignal Reporter Assays (GFP)

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Transcription Factor</th>
<th>Pathway</th>
<th>Transcription Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino Acid Deprivation</td>
<td>ATF4/ATF3/ATF2</td>
<td>MAPK/ERK</td>
<td>Elk-1/SRF</td>
</tr>
<tr>
<td>Androgen</td>
<td>Androgen Receptor</td>
<td>MAPK/JNK</td>
<td>AP-1</td>
</tr>
<tr>
<td>Antioxidant Response</td>
<td>Nrf2 &amp; Nrf1</td>
<td>MEF2</td>
<td>MEF2</td>
</tr>
<tr>
<td>ATF6</td>
<td>ATF6</td>
<td>c-myc</td>
<td>Myc/Max</td>
</tr>
<tr>
<td>C/EBP</td>
<td>C/EBP</td>
<td>NF ( \kappa ) B</td>
<td>NF ( \kappa ) B</td>
</tr>
<tr>
<td><strong>cAMP/PKA</strong></td>
<td><strong>CREB</strong></td>
<td>Notch</td>
<td>Notch</td>
</tr>
<tr>
<td>Cell Cycle</td>
<td>E2F/DP1</td>
<td>Oct4</td>
<td>Oct4</td>
</tr>
<tr>
<td>DNA Damage</td>
<td>p53</td>
<td>PI3K/AKT</td>
<td>FOXO</td>
</tr>
<tr>
<td>EGR1</td>
<td>EGR1</td>
<td>PKC/Ca++</td>
<td>NFAT</td>
</tr>
<tr>
<td>ER Stress</td>
<td>CBF/NF-Y/YY1</td>
<td>PPAR</td>
<td>PPAR</td>
</tr>
<tr>
<td>Estrogen</td>
<td>ER</td>
<td>Retinoic Acid</td>
<td>RAR</td>
</tr>
<tr>
<td>GATA</td>
<td>GATA</td>
<td>SP1</td>
<td>SP1</td>
</tr>
<tr>
<td>Glucocorticoid</td>
<td>GR</td>
<td>STAT3</td>
<td>STAT3</td>
</tr>
<tr>
<td>Heat Shock Response</td>
<td>HSF</td>
<td>TGF ( \beta )</td>
<td>SMAD2/3/4</td>
</tr>
<tr>
<td>Heavy Metal Stress</td>
<td>MTF1</td>
<td>VDRE</td>
<td>Vitamin D Receptor</td>
</tr>
<tr>
<td>Hedgehog</td>
<td>GLI</td>
<td>Wnt</td>
<td>TCF/LEF</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>HIF-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I Interferon</td>
<td>STAT1/STAT2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interferon Gamma</td>
<td>STAT1/STAT1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Cignal Reporter Assays: Two Modalities

Dual-luciferase Format
- Quantitative endpoint 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)
<table>
<thead>
<tr>
<th>Pathway</th>
<th>Transcription Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Cycle</td>
<td>E2F/DP1</td>
</tr>
<tr>
<td>DNA Damage</td>
<td>p53</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>HIF</td>
</tr>
<tr>
<td>MAPK/ERK</td>
<td>Elk-1/SRF</td>
</tr>
<tr>
<td>MAPK/JNK</td>
<td>AP1</td>
</tr>
<tr>
<td>c-Myc</td>
<td>Myc/Max</td>
</tr>
<tr>
<td>NFkB</td>
<td>NFkB</td>
</tr>
<tr>
<td>Notch</td>
<td>RBP-Jk</td>
</tr>
<tr>
<td>TGF β</td>
<td>SMAD2/SMAD3/SMAD4</td>
</tr>
<tr>
<td>Wnt</td>
<td>TCF/LEF</td>
</tr>
</tbody>
</table>
How Does a Cignal Finder Array Work?

1. Add SureFECT™ Transfection Reagent and Test Nucleic Acids
2. Seed Cells for Reverse Transfection
3. Treat & Analyze Phenotype with a Cell-based Assay
4. Cignal™ Finder 10-Pathway Reporter Array (Plate Format)

Focus on Your Pathway™
Topics to be Covered

• Who is SABiosciences?
• Utility of the Cignal Reporter Assays
  • Challenges Associated with Cell-Based Assays
  • Solutions Cignal Reporter Assays Provide
• How Cignal Reporter Assays Work
  • Cignal Pathway Reporters Product Family
  • Dual-Luciferase Assays
  • Luciferase Engineered for High Performance
  • Optimized Transcriptional Regulatory Elements (TREs)

• How YOU Can Use Cignal Reporter Assays
  • Performance and applications data
Cignal Applications

- **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”?
Cignal Application: RNA Interference

![Graph showing Relative Luciferase Units for different conditions: NC, NC siRNA, p53, p53 siRNA, and p53 siRNA. The graph indicates a significant increase in relative luciferase units for p53 siRNA compared to other conditions.]
Cignal Applications

- **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”?

**Focus on Your Pathway™ by SABiosciences**
Cignal Application: Functional Proteomics

![Graph showing luciferase units with different conditions](image_url)

**Relative Luciferase Units**

<table>
<thead>
<tr>
<th>Cignal Reporter</th>
<th>Protein</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>None</td>
<td>25 ng/µl huTGF β1</td>
</tr>
<tr>
<td>NC</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>SMAD</td>
<td>None</td>
<td>2.5 ng/ml huTGF β1</td>
</tr>
<tr>
<td>SMAD</td>
<td>25 ng/ml huTGF β1</td>
<td>25 ng/ml huTGF β1</td>
</tr>
</tbody>
</table>

**Focus on Your Pathway™**
Cignal Applications

• **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”?
Cignal Application: Small Molecule Studies

- Cignal Reporter: CRE
- Small Molecule: None, 0.1µM Forskolin, 1µM Forskolin, 5µM Forskolin, 10µM Forskolin

The graph shows the relative luciferase units for different concentrations of forskolin with varying Cignal Reporter and Small Molecule conditions.

Focus on Your Pathway™
Fluorometry

Fluorescent Microscopy

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

p53 Pathway Interaction Study

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

**Approach:**
Carry out a p53 RNA interference experiment with multiple cancer-relevant Cignal Reporter Assays.
Signal Transduction Cascade
A → B → C → D

(~40 proteins)
Cignal Reporters: Constructing Signaling Networks
Post-Genomics Research Challenge: Pathway Interactions?

- Wnt Signaling
- NFkB Signaling
- E2F Signaling
- MAPK/JNK Signaling
- Hypoxia Signaling
- TGF β Signaling
- Myc Signaling
- Notch Signaling
# Cignal Finder Cancer Array: p53 RNAi Study

<p>| | | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p53 Cignal Reporter</td>
<td>2</td>
<td>RBP-Jk Cignal Reporter</td>
<td>3</td>
<td>TCF/LEF Cignal Reporter</td>
<td>4</td>
<td>E2F Cignal Reporter</td>
<td>5</td>
<td>AP1 Cignal Reporter</td>
<td>6</td>
<td>SRE Cignal Reporter</td>
</tr>
<tr>
<td>7</td>
<td>SMAD Cignal Reporter</td>
<td>8</td>
<td>NFkB Cignal Reporter</td>
<td>9</td>
<td>Myc Cignal Reporter</td>
<td>10</td>
<td>HIF Cignal Reporter</td>
<td>11</td>
<td>Cignal Negative Control</td>
<td>12</td>
<td>Cignal Positive Control</td>
</tr>
</tbody>
</table>

- **p53 siRNA**
- **Neg. control siRNA**
Results: Cancer Array Application Study

Impact of p53 siRNA Treatment

Fold Change in Pathway Activation

Cignal Pathway Reporter
Post-Genomics Research Challenge: Pathway Interactions?

- p53 Signaling
- Hypoxia Signaling
- MAPK/ERK Signaling
- Notch Signaling
Topics to be Covered

• Who is SABiosciences?
• Utility of the Cignal Reporter Assays
  • Challenges Associated with Cell-Based Assays
  • Solutions Cignal Reporter Assays Provide
• How Cignal Reporter Assays Work
  • Cignal Pathway Reporters Product Family
  • Dual-Luciferase Assays
  • Luciferase Engineered for High Performance
  • Optimized Transcriptional Regulatory Elements (TREs)

• How YOU Can Use Cignal Reporter Assays
  • Performance and applications data
Summary: Cignal Reporter Assay System

- **Breadth of Pathways and Research Applications**
  - 35 Pathway-Focused Reporter Assays
  - 4 Application-Specific Cignal Finder 10-Pathway Arrays

- **Performance**
  Exceptional Sensitivity, Specificity, Signal-to-Noise Ratio, and Reproducibility

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

- **Dual Modalities**
  - Dual-luciferase quantitative endpoint assays
  - GFP dynamic live cell assays with single cell resolution
Cignal Reporter Assays

Simple and Robust Signal Transduction Assays

Questions?
Contact Technical Support 9 AM – 6 PM Eastern M – F
Telephone: 888-503-3187;
Email: support@SABiosciences.com