SYGNATURE DISCOVERY

CELL LINE GENERATION

# Delivering Custom Cell-Based Tools for Drug Discovery

# Delivering Quality and Performance for Your Drug Discovery Research

## Supporting Your Research

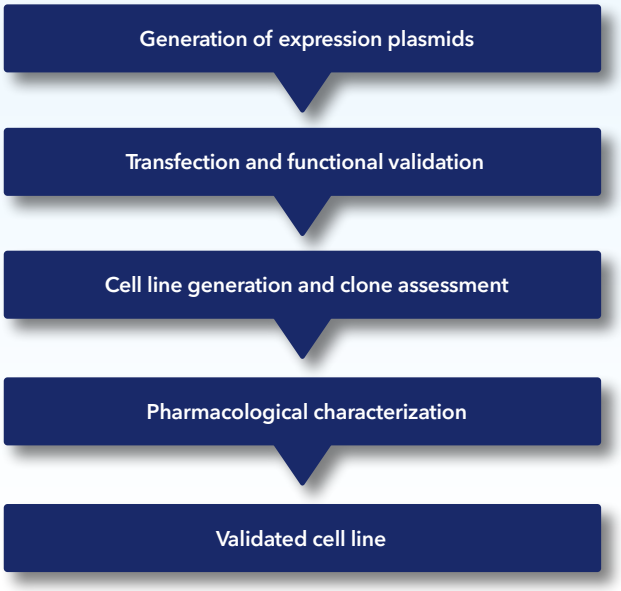
For over two decades, Sygnature Discovery's cell biology experts have excelled in creating high-quality recombinant cell lines and robust assay systems to meet the demands of drug discovery. Our expertise spans challenging targets such as ion channels, receptors and transporters, as well as complex reporter systems and tools to study protein-protein interactions and target degradation, ensuring we meet all our clients' research needs.

Drug Discovery research heavily relies on cell-based assay systems to investigate cellular functions and processes, and to help understand the effect of modulating specific molecular targets.

Challenges associated with the use of primary cultures, such as availability, scalability, and cost, make recombinant cellular systems indispensable for drug discovery. High quality recombinant cell lines provide robust, reliable data and support large-scale high throughput screening, as well as enabling downstream medicinal chemistry-driven cascades due to their excellent reproducibility.

Sygnature Discovery excels in providing fully validated, stable cell lines and assay systems specifically tailored to meet the complex requirements of drug discovery, supporting target validation through to hit identification and lead optimization.

## Cell Line Generation Overview




**Stable, monoclonal cell lines provide consistent, sustained protein expression essential for long-term applications such as high-throughput screening, eliminating the need for time-consuming transfections required when using transient systems. By generating monoclonal lines with uniform expression levels, screening and lead optimization campaigns achieve greater reproducibility, facilitating more reliable data generation.**

Throughout the cell line generation process, individual clonal isolates are characterized in terms of health, functionality and pharmacology to ensure the optimal clone is selected for your needs.

# Vector Design

## Choice of Promoter

The choice of promoter plays a crucial role in driving reliable gene expression. While the CMV promoter is commonly utilized due to its ability to induce high expression, if lower expression levels are desired, then alternative promoters should be considered to drive more physiologically relevant expression. Additionally, host cell factors should also be taken into account to avoid the risk of cell-specific promoter silencing.

## Antibiotic Resistance

Incorporating antibiotic resistance genes is essential for the generation of stable cell lines. Mammalian resistance markers such as neomycin, hygromycin, zeocin, blasticidin, and puromycin are commonly used to isolate target-expressing clones. An important consideration is how to strategically design the optimal combination of resistance genes to ensure continued cell health when expressing multiple proteins of interest in a single expression system.

## Protein Co-Expression

If your studies require expression of multiple proteins, different strategies are available to ensure robust expression of each gene. Factors to consider include appropriate protein to protein stoichiometry as well as refining expression levels to deliver the optimal assay window over background, all of which can be controlled using appropriate expression vectors, including those designed to express multiple proteins from a single plasmid.

## Expression Regulation

Not all recombinant cell systems benefit from constitutive target expression. In some cases, target over-expression can have toxic consequences for the cell, while in other cases it may be beneficial to assess the same cell system in the presence and absence of a specific protein. Inducible systems offer precise control over gene expression levels, overcoming cytotoxicity challenges by silencing protein expression when the target is not required.

## Surface Expression

For proteins that need to be targeted to the cell surface, incorporating specific tagging elements can enhance plasma membrane expression. These tags can act as chaperones to facilitate surface expression and localization, or can stabilize expression at the cell surface, enhancing the target's functionality and utility in a cellular assay system.

## Detection Tags

Availability of suitable antibodies to detect target expression is a key component of clone selection during cell line generation. In cases where specific antibodies for protein detection are unavailable, incorporation of tags can facilitate detection and aid the characterization processes. Detection tags also provide versatile options for protein purification, enabling isolation for downstream cell-free applications.

## Choosing the Right Host Cell Line

Selecting the right host cell line for downstream cell-based assays is a crucial step when planning your cell line generation strategy. A variety of commonly used host cells are available including HEK and CHO amongst others. However, it is also important to consider whether the chosen host cell possesses the appropriate signalling components required for functionality of the target protein. Possessing the appropriate cellular machinery can be key to successful cell line generation when studying targets associated with cell type specific signalling pathways. Reviewing historical literature can help guide this selection process alongside knowledge of the natural target-associated tissue environment.

## Inducible Expression Control

Constitutive expression systems enable continuous protein levels. However, sustained expression may not be suitable for all targets, especially those which are toxic to cells when expressed continuously.

Inducible expression systems on the other hand, offer more precise control over protein expression levels. These engineered systems allow protein expression to be turned on and off, thus protecting the cell from long-term toxic over-expression. Inducible expression systems can also be used to fine tune the level of target expression, providing distinct advantages in terms of defining the relative expression levels between two proteins and allowing for fine control of protein expression ratios for optimal assay performance.

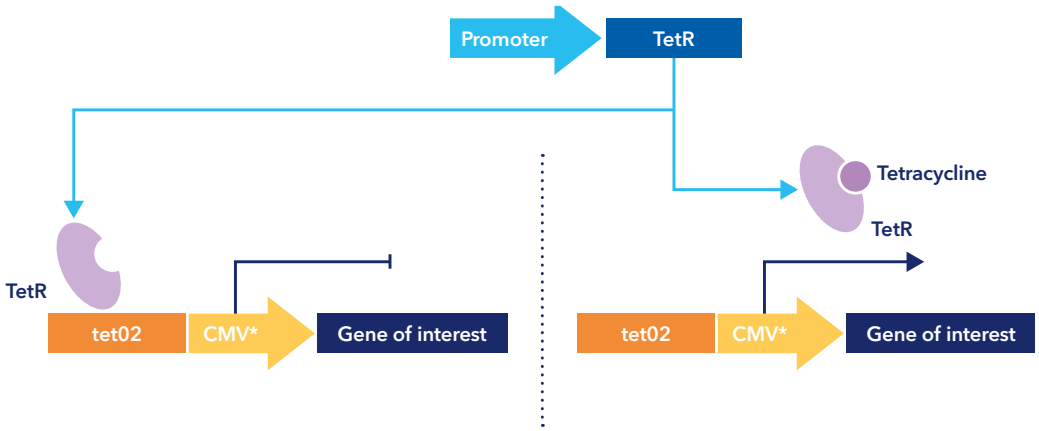



**Figure 1**
Tetracycline controlled regulation of gene expression

# Validating Target Expression and Functionality

Following transfection and monoclonal isolation, antibiotic-resistant clones are screened for presence of transcript and protein expression using a combination of real-time quantitative PCR and western blot, while flow cytometry is used to assess protein surface expression and measure cell line homogeneity. Selected clones are then assessed in a relevant functional assay to identify those displaying optimal signal to noise ratio and to ensure target possesses the correct pharmacology.

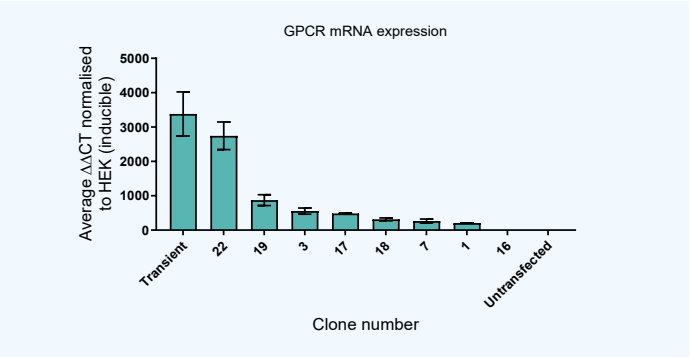



Figure 2
Target transcription levels determined using real-time quantitative PCR.

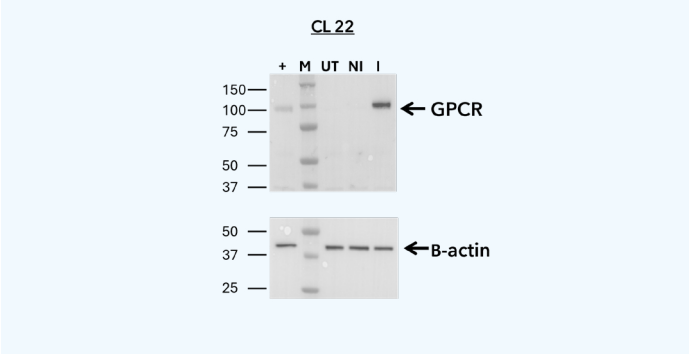



Figure 3
Western blot verification of target protein expression. Target protein detected in the tetracycline induced (I) sample only.

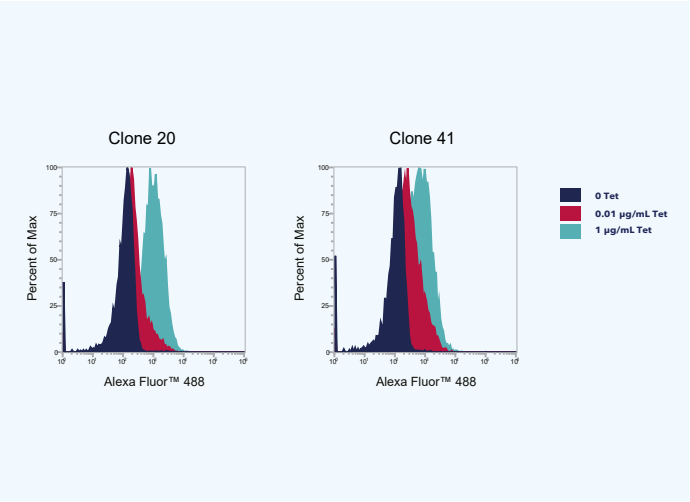



Figure 4
Assessment of target cell surface expression and clone homogeneity using flow cytometry.

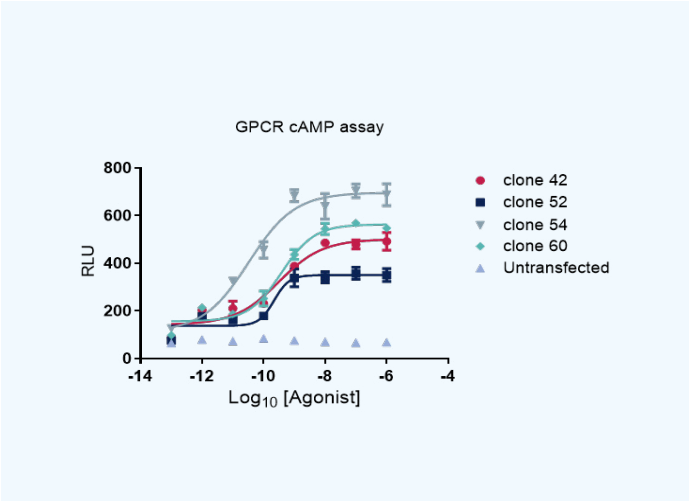


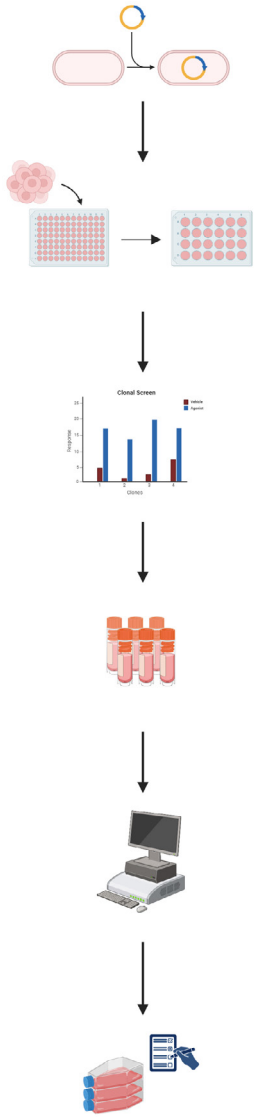

Figure 5
Functional assessment of target-expressing clones in a cell-based cAMP accumulation assay.

## Validation Platforms

Sygnature Discovery's cell line generation capabilities are complemented by wide array of advanced assay platforms, ensuring the optimal clone is selected for your downstream functional endpoints.

| | |
|---|---|
| **Radioligand Binding** | Quantification of receptor-ligand interactions with high specificity and sensitivity, providing crucial data on binding affinity and kinetics. |
| **Flow Cytometry (FACS)** | Utilizing fluorescent markers, FACS assays facilitate the analysis of both total and cell surface expression, enabling detailed profiling of cell populations and receptor expression levels. |
| **Calcium Mobilization** | Designed to measure intracellular calcium flux, these assays are ideal for studying GPCR and ion channel function as well as other calcium-dependent cellular processes. |
| **cAMP Measurements** | Optimized assays to detect changes in cAMP levels, providing insights into cell signalling, target receptor activity and intracellular signalling pathways. |
| **Reporter Assays** | Enable assessment of target activation and signalling cascades, making them ideal for studying signal transduction and regulatory elements associated with a wide range of pathways. |
| **Membrane Potential** | Measure changes in membrane potential, crucial for understanding ion channel activity and cellular excitability. |
| **Thallium Flux** | Designed to measure potassium flux, ideal for high-throughput screening against potassium-conducting ion channels. |
| **Electrophysiology** | State-of-the-art manual and high-throughput automated electrophysiology assays provide detailed analysis of ion channel function, delivering high-resolution data at an unparalleled scale. |

# Cell Line Generation




## 1 Plasmid Transfection

Transfect host cells with recombinant plasmids containing the target gene and an antibiotic resistance marker. Post-transfection, antibiotic selection eliminates non-transfected cells, ensuring only transfected cells survive and proliferate.

## 2 Cell Pool Generation and Expansion

Transfected cells, maintained in antibiotic selection media, form clones in 96-well plates. Limiting dilution used to ensure colonies are derived from single cell per well. Cells are kept in antibiotic selection media and clones are expanded for screening.

## 3 Clone Screening

The expanded clonal populations undergo thorough target-specific functional screening (e.g. electrophysiology, calcium assays, membrane potential assays, luciferase assays, protein expression analysis, flow cytometry).

## 4 Low Passage Stock Generation

Low passage stocks are created from optimal clones. Clones are re-initiated from frozen stocks to confirm viability and target expression.

## 5 Functional Assessment

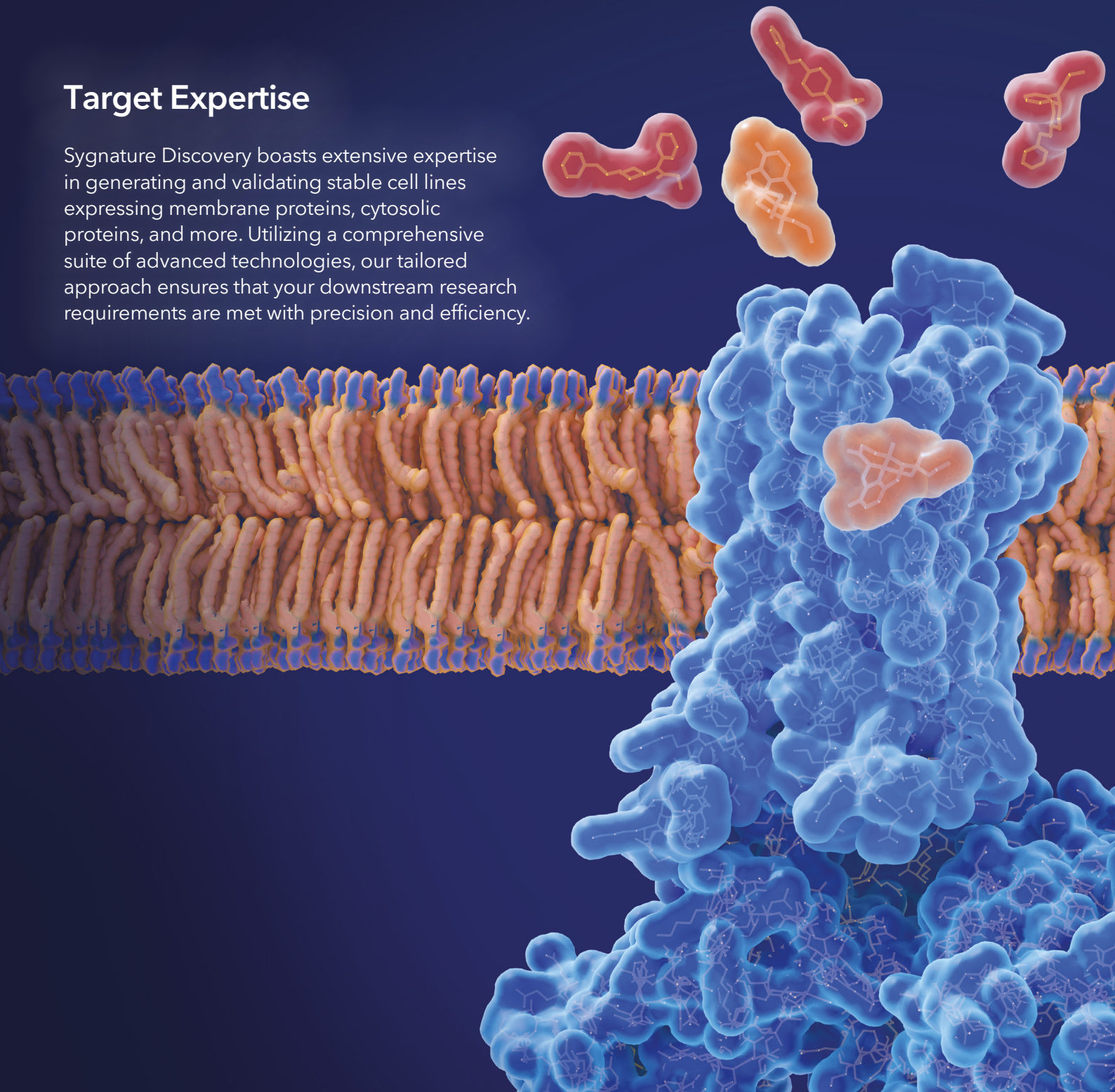
Selected clones undergo pharmacological assessment to validate response to specific stimuli. Top-performing clones are identified based on criteria such as cell growth rate and assay performance. Top clones are then chosen for master stock generation.

## 6 Quality Check

Master stocks undergo rigorous quality checks for integrity, stability and performance reproducibility.

# Target Expertise

Sygnature Discovery boasts extensive expertise in generating and validating stable cell lines expressing membrane proteins, cytosolic proteins, and more. Utilizing a comprehensive suite of advanced technologies, our tailored approach ensures that your downstream research requirements are met with precision and efficiency.



## Ion Channels

Ion channels are essential membrane proteins that facilitate the flow of ions across cell membranes, regulating electrical activity and maintaining cellular homeostasis. At Sygnature Discovery, we have successfully designed, generated and validated over 150 recombinant ion channel cell lines and assays. Our extensive portfolio supports a wide range of ion channel research applications, from high-throughput screening to off-target selectivity studies.

### Example - BK Channels

A comprehensive suite of cell lines expressing BK channel subtypes has been generated to enable evaluation of calcium sensitivity, kinetics and compound modulation, ensuring robust reliable data for BK channel drug discovery. Clonal isolates were characterized by high-throughput automated electrophysiology to determine the best clones for downstream studies.

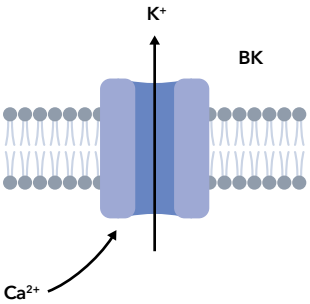



**Figure 6**
Schematic diagram illustrating $Ca^{2+}$ activation of BK channel.

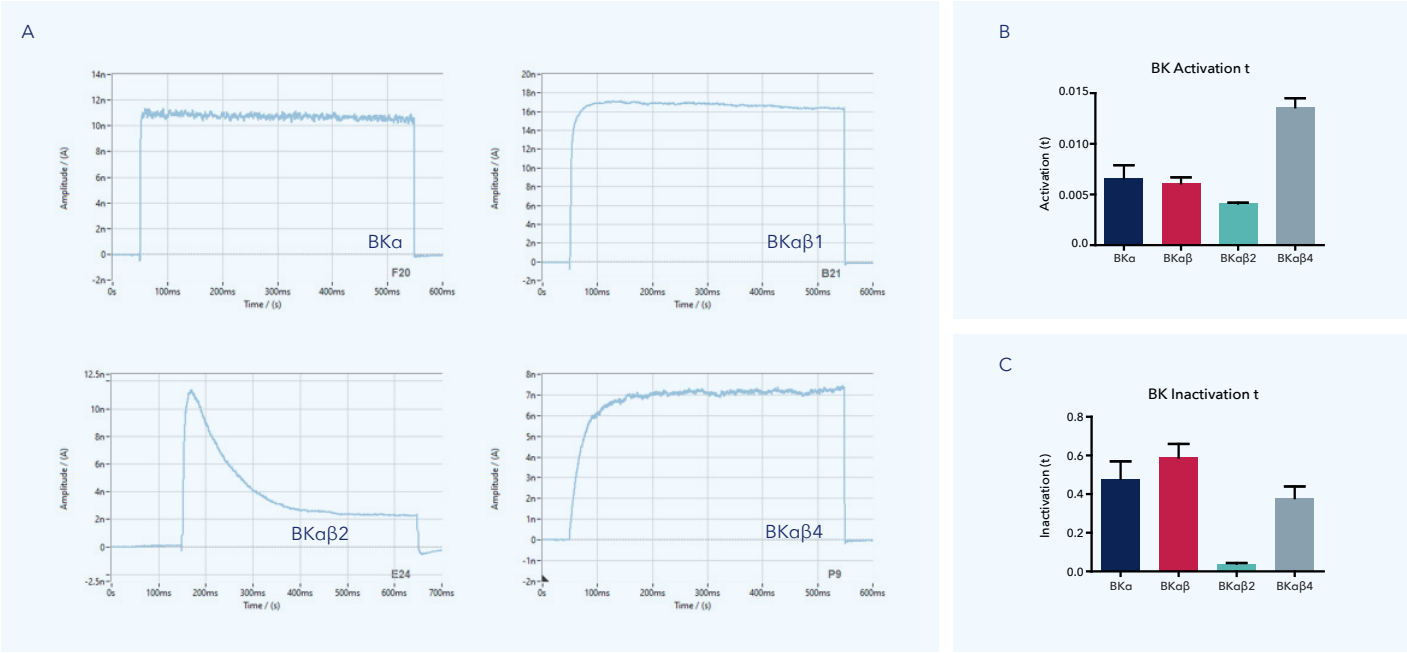



**Figure 7**
BK Channel Kinetics. (A) Representative current traces of the BK subtypes in the presence of 10 µM internal free $Ca^{2+}$ monitored using a peak pulse. The activation (B) and inactivation (C) tau value of each BK subtype is shown in the presence of 10 µM internal free $Ca^{2+}$. As expected, the BKαβ4 subtype displayed a slower activation rate and BKαβ2 exhibited the fastest inactivation rate.

## GPCRs

G protein-coupled receptors (GPCR's) and their associated signalling cascades regulate numerous physiological processes in the body. G-proteins are critical components of these signalling systems, helping transmit and amplify stimuli from outside the cell to the interior. In recombinant assay systems, co-expression of the optimal G-protein can significantly enhance downstream assay performance while chimeric G-proteins enhance coupling and can be used to drive signalling via desired pathways, facilitating endpoint readouts of choice and streamlining screening cascades.

Sygnature Discovery has generated a comprehensive panel of G-protein constructs encoding both wild-type and chimeric G-proteins to aid design of robust GPCR assay systems. Whether you are trying to identify the optimal receptor/G-protein combination or drive signalling via a specific pathway for downstream assay convenience.

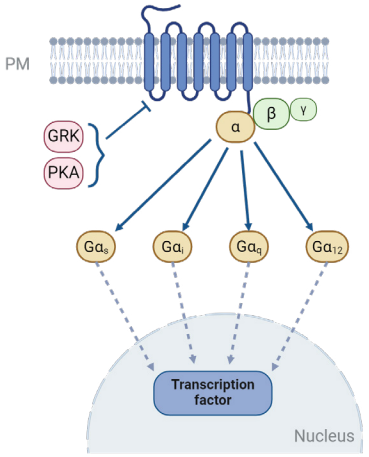



**Figure 8**
Schematic diagram illustrating common GPCR signalling pathways.

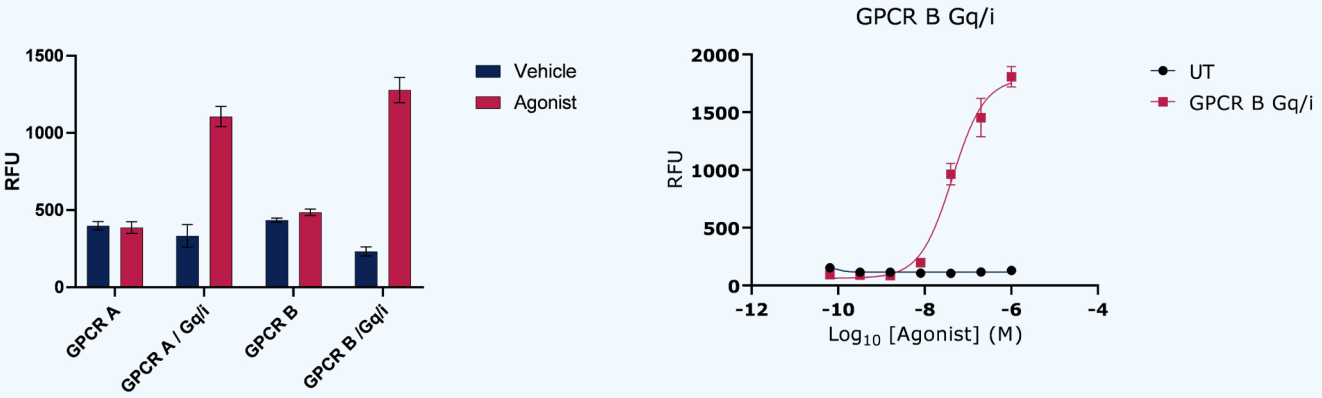



**Figure 9**
Gi-coupled GPCRs can be forced to signal via Gq pathway using chimeric G proteins, enabling high-throughput screening using calcium mobilization assays.

## Transporters

Human Solute Carrier (SLC) transporters are specialized proteins responsible for transporting molecules, ions, and other substances across the cell membrane, playing a crucial role in maintaining cellular homeostasis. These transporters regulate the internal environment of the cell by controlling nutrient uptake, waste elimination, and cell signalling processes through their selective permeability.

At Sygnature Discovery, our cell line generation group excels in developing recombinant transporter cell lines and assays to support drug discovery research. Our extensive knowledge enables development of functional cell lines and assay systems designed to assess transporter activity, facilitating the identification of novel drug candidates and supporting lead optimization. Utilizing advanced SLC transporter assay technologies including fluorescence-based HTS and solid-supported membrane electrophysiology, we offer customizable services tailored to meet your specific research needs, ensuring comprehensive and reliable outputs for your discovery research.

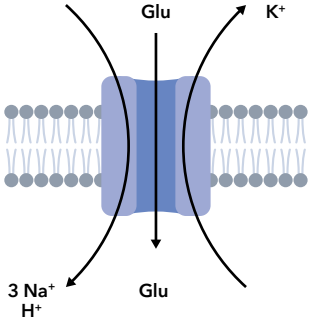



**Figure 10**
Schematic diagram illustrating the ion-flux coupling stoichiometry for glutamate transporters.

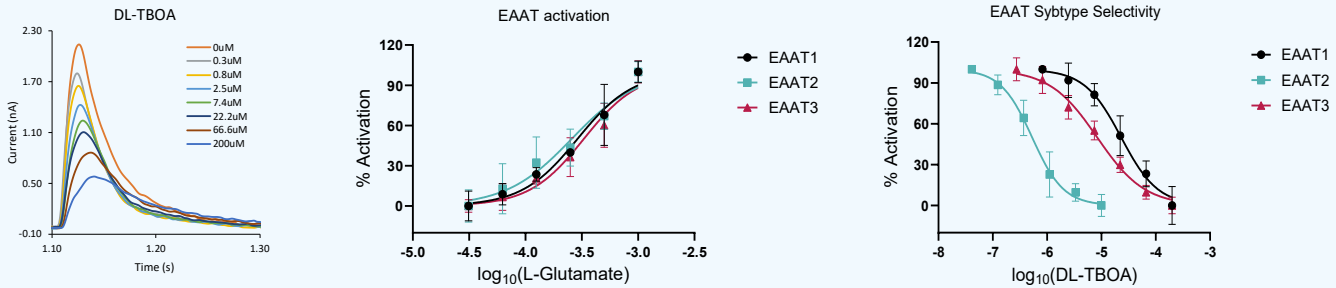



**Figure 11**
Example data generated using Solid-Supported Membrane Electrophysiology. (Left) Current traces showing concentration-dependent inhibition of EAAT2 activity by DL-TBOA; (Middle) Concentration-dependent activation of EAAT1, EAAT2, and EAAT3 by L-Glutamate; and (Right) Concentration-response curves showing inhibition of EAAT1, EAAT2, and EAAT3 activity by the reference inhibitor DL-TBOA.

# Technologies

## Reporter Systems

Sygnature Discovery has developed a diverse range of reporter constructs and assays designed to facilitate complex GPCR and other receptor signalling pathway studies. These reporters, when integrated into stable cell lines expressing your gene of interest, enable measurement of pathway activation using easily quantifiable reporter genes such as luciferase. Our expert team specialises in customizing reporter assay solutions tailored to your specific research needs. By combining the expression of your target gene with these advanced reporter constructs, we provide powerful tools to help elucidate intricate signalling mechanisms and advance your research goals.

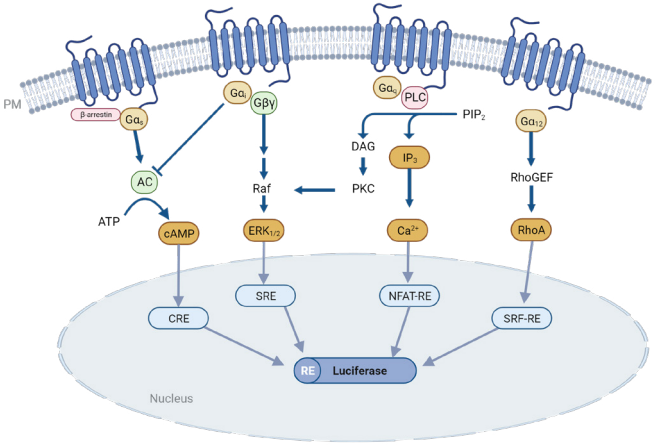



**Figure 12**
Schematic diagram illustrating GPCR signalling pathways and associated reporters.

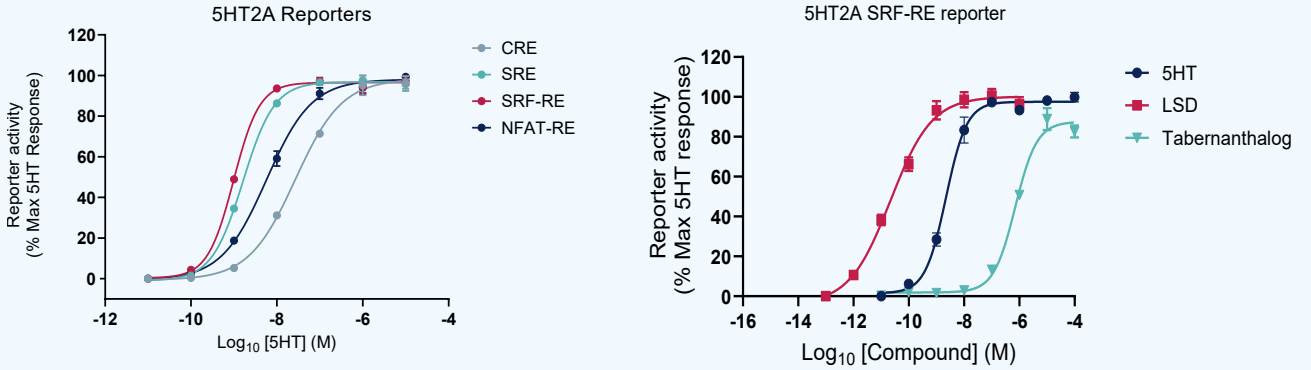



**Figure 13**
Effect of ligand against target receptor assessed using a range of reporter vectors to investigate differential signalling.

## Protein-Protein Interactions

To facilitate the study of protein-protein interactions and the discovery of modulators of these interactions, Sygnature Discovery employs cutting-edge technologies to create robust stable cell lines and assay systems capable of real-time measurement of protein interactions within live cells. Using a variety of technologies, our scientists enable precise and dynamic monitoring of protein-protein interactions, providing invaluable insight into the intricate mechanisms governing cellular signalling.

By leveraging such technologies, Sygnature Discovery's expertise in protein interaction is ideally suited to the study of biased GPCR signalling. As well as canonical signalling via G-proteins, GPCRs also engage in signalling via non-canonical routes, for example through β-arrestin recruitment. Generation of assay tools to monitor β-arrestin recruitment by GPCRs enables the identification of novel biased signalling modulators, presenting opportunities to selectively modulate specific signalling pathways downstream of a single receptor target.

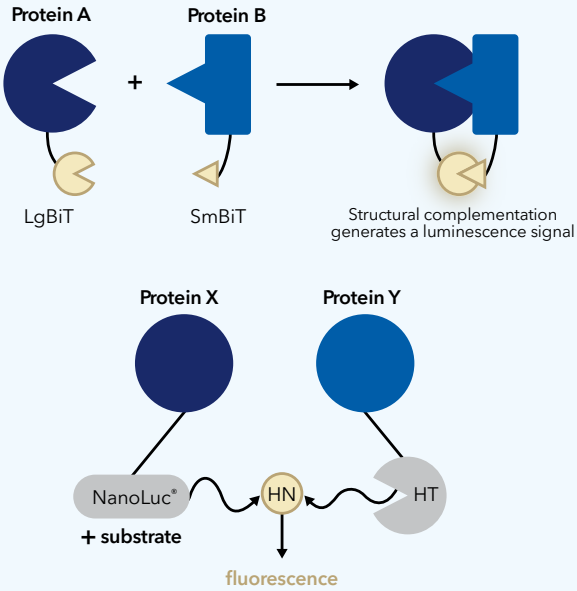



**Figure 14**
Protein-protein interactions can be measured using the NanoBiT or NanoBRET assay systems. The NanoBiT technology (top) relies on LgBiT or SmBiT tagged proteins of interest, upon interaction, nanoluciferase is generated and signal detected. The NanoBRET assay (bottom) utilizes proteins of interest tagged with nanoluciferase or halotag. When in close proximity, and in the presence of substrate, protein interaction can be detected.

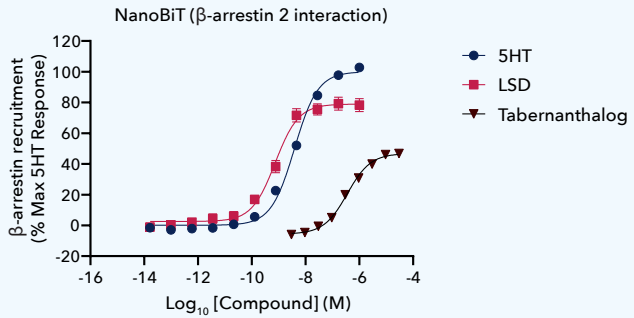



**Figure 15**
Concentration-dependent effect of various tool compounds on recruitment of β-arrestin by 5HT2A receptors using NanoBiT protein interaction technology.

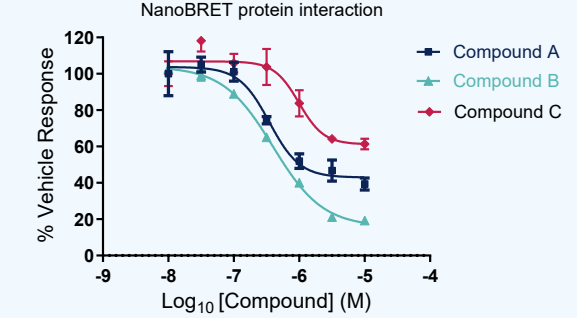



**Figure 16**
The effect of compounds on protein-protein interactions assessed using NanoBRET assay.

## Receptor Internalisation

Receptor internalization is a key regulatory mechanism for nearly all GPCRs. Initially thought to prevent excessive cell surface signalling by reducing receptor availability, it is now understood to also enable 'second wave' signalling from intracellular membranes.

Ligand-induced receptor internalisation can be measured using recombinant cell lines expressing the receptor of interest fused with a small detection tag, enabling distinction between cell surface and intracellular receptor localisation. Sygnature Discovery's assay development team utilize such technologies to create model system for measuring receptor internalization as well as studying trafficking of proteins from intracellular sites to the plasma membrane.

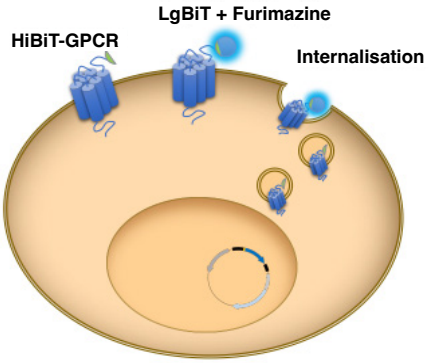



Figure 17
Using the Nano-Glo® HiBiT Extracellular Detection System, receptors tagged with the small HiBiT tag can be quantified on the cell surface and compared to total cellular expression. Ligand-induced internalisation results in a shift in ratio between surface and total target expression.

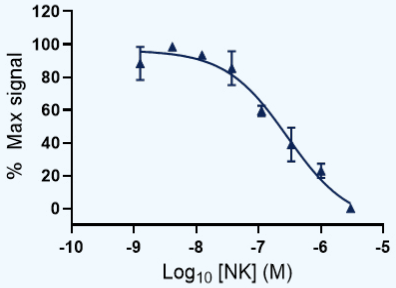



Figure 18
HiBiT tagged GPCR expressing cell line displaying quantifiable, ligand-induced receptor internalization.

## Protein Degradation

In addition to monitoring receptor internalization, these technologies can be used to measure protein degradation. By generating a stable cell line expressing a protein of interest fused to a quantifiable tag, target expression can be measured in the presence and absence of putative degraders to identify novel strategies for targeted protein degradation.

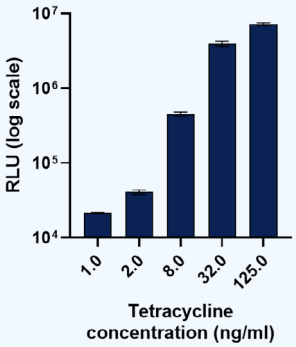



Figure 19
Tuneable expression level is critical for the successful development of cell-based degrader assays, where lower expression levels may enhance quantification of protein degradation.

## Biosensors

Molecular biosensors are designed for sensitive detection of second messengers, allowing for continuous, real-time measurement of intracellular signalling events in live cells. Ideally suited for high-throughput formats, these biosensors provide innovative methods for studying GPCR signalling, including cAMP accumulation and calcium mobilization.

Sygnature Discovery leverages its expertise in cell line generation and signalling to create robust assay systems that maximize the power of these biosensors. By employing validated, high-throughput compatible technologies, we provide advanced tools that significantly enhance your research.

Our expertise in cell line generation combined with our broad assay development skills enables generation of assay systems to assess second messenger signalling in real-time. These biosensor assay systems deliver precise, dynamic data on second messengers, offering valuable insights into cellular signalling pathways.

With these advanced biosensor technologies, Sygnature Discovery is helping researchers gain unprecedented access to the intricate dynamics of cellular signalling.

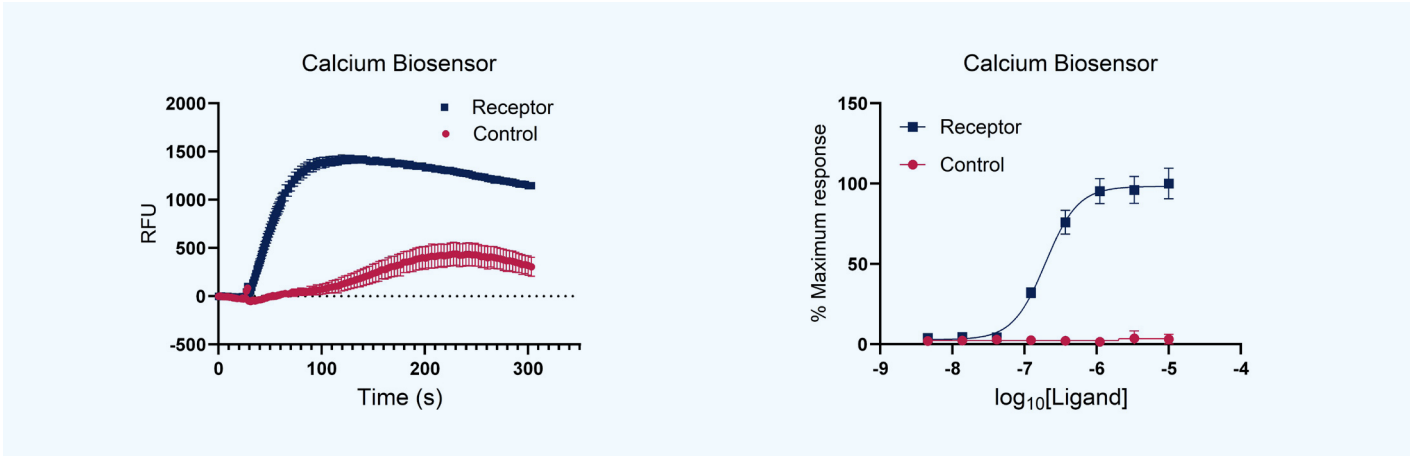



**Figure 20**
Measurement of calcium flux using a fluorescent biosensor. Concentration-dependent effect of ligand on calcium accumulation after addition of agonist (left). $EC_{50}$ for agonist generated by plotting maximum fluorescence values (right).

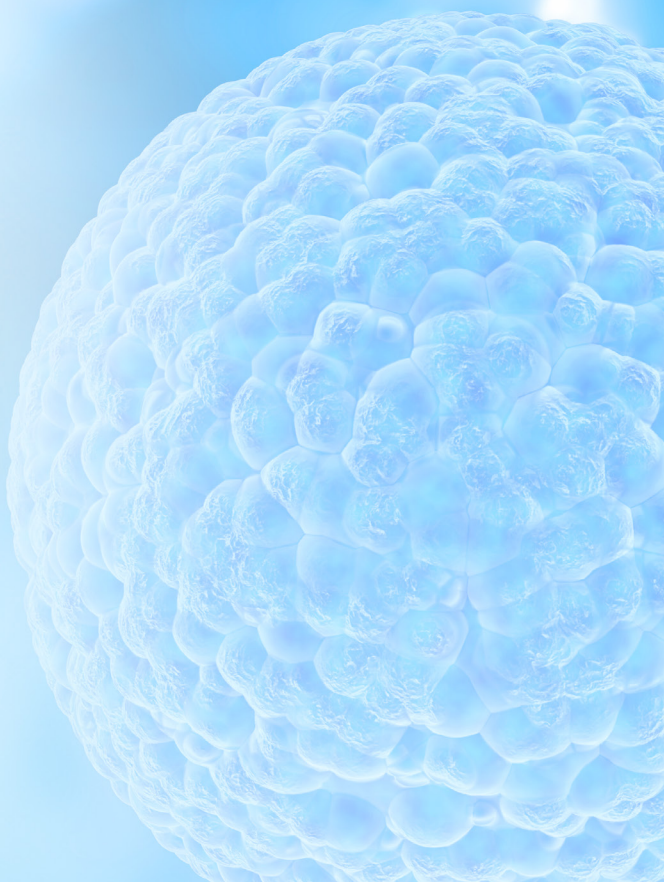
# Delivering Quality and Performance to Your Drug Discovery Research

## Why Choose Sygnature Discovery for Custom Cell Line Generation?

Sygnature Discovery's cell biology team has been generating high quality recombinant cell lines for over 20 years and has amassed extensive expertise in many challenging target classes, meticulously analysing the data from many hundreds of clones per target. We provide you with the best reagents possible, speeding up your research and giving greater confidence in your data.

Our knowledge of expression systems and host cell types, together with extensive troubleshooting expertise, is of paramount importance to help achieve your goals. The challenges our customers present us with and the strategies we have devised to overcome these, have further enhanced our expertise and reputation as we continue to deliver the most intricate recombinant cellular systems to support drug discovery.

Our collaborative approach ensures you are involved in all critical decisions as we advance, while also allowing you to concentrate on other aspects of your research. With unparalleled expertise in cell line generation, we take the stress out of your reagent development plans and quickly drive forward your drug discovery research.




sygnaturediscovery.com