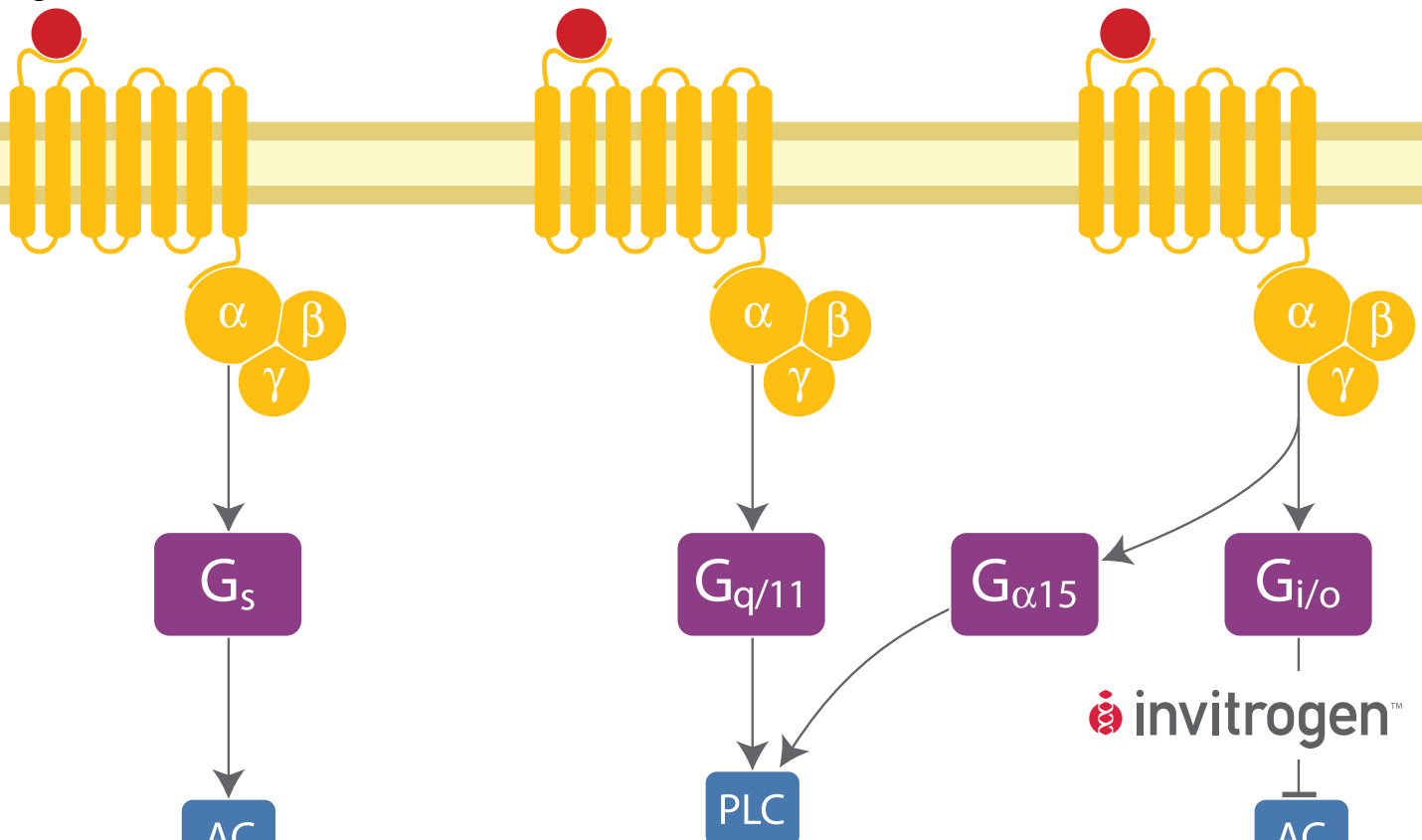




Couple your GPCR profiling needs with our expertise

SelectScreen™ Cell-Based GPCR Profiling Service

Ligand





SelectScreen™ Cell-Based GPCR Profiling Service

- Enhance binding data utilizing functional, cell-based GPCR assays
- Determine activity of disease-relevant GPCR targets following exposure to drug candidates or other stimuli
- Address functional selectivity with beta-arrestin recruitment and second-messenger GPCR assay formats

When assessing your compound's effects on the activity of various GPCR targets, binding data tell only part of the story. Increase the value of your GPCR profiling projects by using Invitrogen's functional SelectScreen™ Cell-Based GPCR Profiling Service. Our GPCR profiling service utilizes functionally active GPCRs with the GeneBLAzer™ second-messenger and Tango™ beta-arrestin recruitment assay technologies to create unique, multidimensional tools to interrogate GPCR biology. The reputation of the SelectScreen™ service was built on rapid delivery of highest-quality data. Faster access to reliable data allows you to move more quickly through compound profiling projects and ultimately speeds your discovery efforts. Ensure that you have an accurate functional interpretation of the effects of your compounds with the SelectScreen™ GPCR Cell-Based Profiling Service.

Built-in quality

All SelectScreen™ services are performed at Invitrogen's Discovery Assays and Services facility. All reagents used in the SelectScreen™ services are manufactured by Invitrogen, ensuring the integrity and quality of the reagents. Furthermore, the cell lines and substrate used during the screening service are available for purchase should you require subsequent screening at your facility. Control or reference compounds are included on every plate for each cell line we assay. Data points are generated in duplicate (n=2), and all services are completely confidential. Our open business model allows for flexible, volume-based project discounts.

Screening expertise

At Invitrogen, we have more than 10 years of experience generating, validating, and screening cell-based assays for HTS, hit-to-lead, and lead optimization. Additionally, the scientists involved in developing and performing the SelectScreen™ services have significant screening expertise from past experience at major pharmaceutical and biotechnology companies. We put this experience to work for you, delivering you highest-quality results.

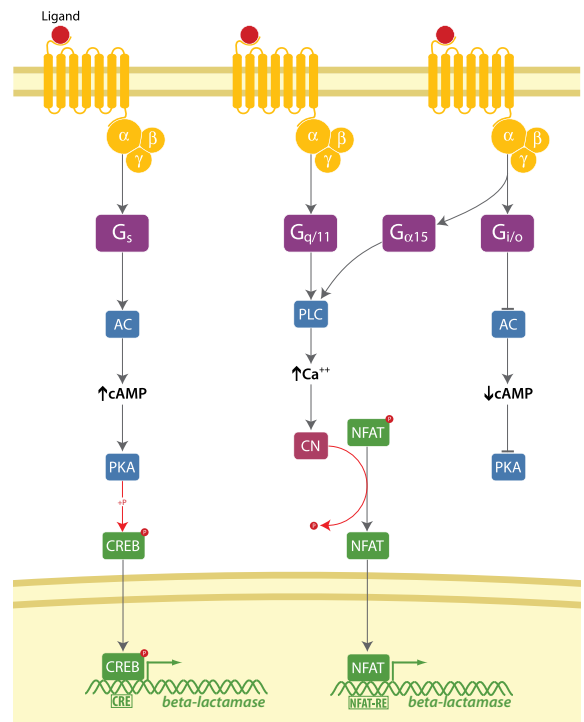


Figure 1—GeneBLAzer® technology utilizes second-messenger signaling pathways leading to CRE or NFAT response elements driving expression of the beta-lactamase gene.

Technology behind the SelectScreen™ service

The SelectScreen™ Cell-Based GPCR Profiling Service utilizes Invitrogen's growing library of functional, validated GeneBLAzer® and Tango™ GPCR cell lines.

GeneBLAzer® and Tango™ GPCR cell-based assays:

- Provide ready-to-screen, ratiometric assays for disease-relevant targets
- Are functionally validated with known agonists and antagonists (when available) to ensure high-quality results every time
- Have Z'-factors of ≥ 0.5 for agonist assays and ≥ 0.4 for antagonist assays

Both of these GPCR signaling technologies activate a stably integrated beta-lactamase (*bla*) reporter gene (Figures 1 and 2). The GeneBLAzer® GPCR cell lines couple through the respective second-messenger signaling cascade upon activation of the receptor (Figure 1). The Tango™ GPCR cell lines circumvent the second-messenger cascade and utilize the beta-arrestin recruitment mechanism within the cells (Figure 2). Upon expression, the beta-lactamase enzyme cleaves the LiveBLAzer™-FRET B/G Loading Substrate to provide a selective and quantitative FRET-based readout of GPCR activity (Figure 3). The rapidly growing panel of targets available in the SelectScreen™ services utilizes cells that are cryopreserved and/or division-arrested to allow for the rapid turn around of your projects. The service provides a rapid, reliable, and sensitive method of analyzing the activity of a wide range of disease-relevant GPCRs upon exposure to drug candidates or other stimuli.

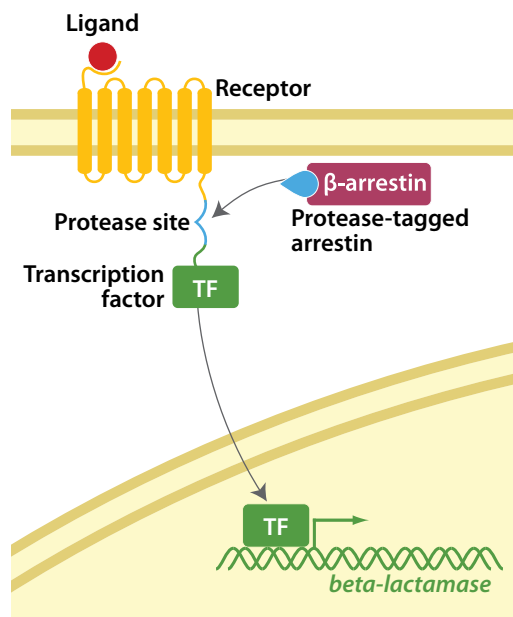


Figure 2—Tango™ technology utilizes cellular beta-arrestin recruitment of a protease tagged beta-arrestin to a receptor engineered with a protease cleavage site, releasing the transcription factor to drive expression of beta-lactamase.

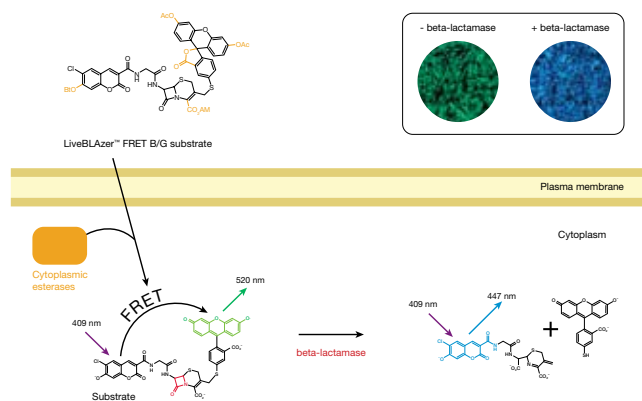


Figure 3—The expression of beta-lactamase in cells causes the cleavage of the lactam ring of the substrate, disrupting the coumarin and fluorescein FRET pair. Cells expressing beta-lactamase will appear blue as the FRET pair is disrupted. Cells not expressing the beta-lactamase reporter will appear green, indicating the FRET pair is intact.

Profile a single receptor or the entire family

You can screen any of the cell lines from Invitrogen's SelectScreen™ GPCR Service panel, which represents a broad selection of disease-relevant, therapeutic areas such as oncology, immunity/inflammation, neuroscience, and cardiology. A comprehensive panel of GPCR assays requires that key GPCR families are represented. Represented in the SelectScreen™ GPCR Cell-Based Profiling Service are several complete GPCR families, including acetylcholine (muscarinic), cannabinoid, melanocortin, opioid, and PACAP receptors. The number of available GPCR cell lines is continually expanding; visit www.invitrogen.com/gpcrprofiling for updates.

Proven performance

To demonstrate the value of functional, cellular GPCR profiling, the SelectScreen™ Cell-Based GPCR Profiling Service was used in several experiments, for which the assays and results are discussed below.

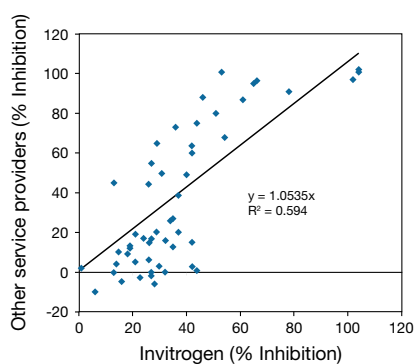


Figure 4—Correlation graph of Invitrogen's SelectScreen™ Cell-Based GPCR Profiling Service to other service providers. A customer provided Invitrogen with a small subset of compounds biased toward selected GPCRs. The percent inhibition values determined using the SelectScreen™ GPCR Service were calculated and plotted against those provided by the customer, determined using a variety of different technologies.

Correlation of data across multiple assay formats

A select number of GPCR targets was screened against several compounds for a client. The percent inhibition from the SelectScreen™ GPCR Profiling Service was compared to data from two different services using either a binding assay or a different functional assay platform (Figure 4). Reasonable correlation between the three different assay formats was observed, with an R^2 value of ~ 0.6 .

Identification and confirmation of 'hits' with a functional GPCR assay

A functional cell-based GPCR screen utilizing our Tango™ beta-arrestin assay technology was conducted. A library of 5,000 compounds was screened in singlicate in agonist mode using our U2OS cells transfected with the opioid receptor mu 1 (OPRM1). As expected, a tight distribution centered around 0% activation was obtained, and compounds with $> 40\%$ activation (a 0.3% hit rate) were identified (Figure 5). Dose-response experiments with the identified hits were performed, resulting in an 88% confirmation rate.

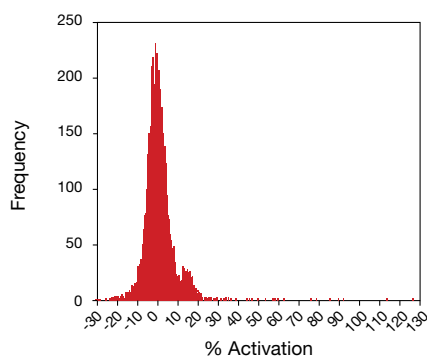


Figure 5—Frequency graph for a small library screen using the SelectScreen™ Cell-Based GPCR Profiling Service. Over 5,000 compounds were screened with an average Z'-factor value of 0.73.

Flexible approach to screening

You can choose from multiple parameters to design the optimal project to meet your screening goals. Options include:

- Screening mode:
 - Single data point/concentration determinations for 40 compounds or more per target (one data point = two wells, $n = 2$)
 - EC_{50}/IC_{50} determinations using 10-point dose-response curves (1/2 log serial dilutions) ($n = 2$)
 - Agonist (% activation) and/or antagonist (% inhibition) mode
 - A starting concentration of your choice
- Compound supply—choose from a wide range of combinations for screening, from a small subset of compounds against multiple cell lines to many compounds on one cell line, such as a library screen
- Turnaround time—you will receive results of the service within 2–3 weeks, depending on the scope of the project
- Validation—counterscreen your “hits” against the parental cell lines for GeneBLAzer® technology to eliminate any nonspecific compound effects

How to order

The SelectScreen™ Cell-based GPCR Profiling Service is straightforward and efficient to use. No subscription or enrollment is necessary.

Projects proceed through the following steps:

- Complete the SelectScreen™ submission form available online at www.invitrogen.com/gpcrprofiling.

- Receive confirmation from Invitrogen that includes the scope of the project and pricing.
- Supply your purchase order number and prepare compounds for submission to Invitrogen.
- Receive your data analysis results as an electronic file for easy upload to your internal database.

The protocol and assay conditions used in the SelectScreen™ services, as well as the compound submission guidelines, are available to view on the corresponding web page for each service.

Need rapid compound generation or medicinal chemistry support?

Looking for access to new compound collections for your library screening project or additional medicinal chemistry support? Invitrogen has partnered with Nanosyn to provide a complete package for clients interested in screening novel compound collections. Nanosyn offers a variety of focused compound libraries of differing sizes that have been preconfigured to be readily screened in the Invitrogen SelectScreen™ Library Screening Service. Nanosyn also has extensive analytical chemistry capabilities to quantitatively evaluate and configure your compounds so we can quickly transfer them into our SelectScreen™ Library Screening and other SelectScreen™ profiling services.

For more information on compound supply, please visit www.nanosyn.com.



Additional SelectScreen™ compound screening services available from Invitrogen

Invitrogen offers a suite of screening and profiling services to support your discovery research:

- SelectScreen™ Kinase Profiling Service—hundreds of kinase assays available
- SelectScreen™ Library Screening Service—screen large library collections against any of Invitrogen’s kinase, nuclear receptor, GPCR, or pathway assays, or an assay of your choice
- SelectScreen™ Cell-Based Pathway Profiling Service—more than 20 cellular pathway assays
- SelectScreen™ Cell-Based Nuclear Receptor Profiling Service—the largest selection of cell-based steroidal and nonsteroidal family nuclear receptor assays
- SelectScreen™ P450 Profiling Service—determine your lead compound’s inhibitory profile against five key CYP450 isozymes
- SelectScreen™ hERG Screening Service—identify compounds that bind the hERG channel
- SelectScreen™ Custom Screening Service—let us build the assay for you and screen your compounds

Learn more at www.invitrogen.com/profilingpartner.