PathHunter™ β-Arrestin Assays as a Universal Tool For Orphan GPCR Analysis
Tom Wehrman PhD, Daniel Bassoni, Neil Charter, PhD, Albert Doan, Chin-Yee Loh, Bill Raab, and Keith R. Olson, PhD
DiscoverRx Corporation, Fremont, CA 94538, USA

Abstract
Orphan GPCRs continue to represent a target class with immense potential for the discovery of novel therapeutic compounds. In spite of the proven track record of GPCRs as drug targets, over 100 non-orphan GPCRs remain to have their cognate ligands identified. Here we present the PathHunter Arrestin assay as a facile screening approach to orphan GPCRs drug discovery. In this system, a small peptide is fused to the GPCR target, and the complementing fragment is fused to β-Arrestin2. Upon activation, the GPCR binds to arrestin for complementation of the enzyme fragment. The activity is detected via a single addition, HTS friendly, chemiluminescent reagent and is analyzed without waiting for reporter gene signals. The assay is performed in the absence of second messenger signaling of the target and does not require the presence of ligands. Reporter gene results associated with other assays and makes it an ideal format for primary HTS screening. DiscoverRx has created an industry leading portfolio of orphan GPCR stable cell lines that are available for surrogate ligand discovery, de-orphanization, and profiling. This presentation will focus on the validation of the PathHunter system for orphan GPCR research and screening, the development of a cell line panel for profiling, and proof data from a focused library that receptors can in fact be de-orphanized with our system. Furthermore, we will present existing examples where orphan GPCRs appear to regulate the function of characterized GPCRs, highlighting the potential for orphan receptors to heterodimerize with other GPCRs and modulate their activities.

Cell-Based PathHunter β-Arrestin Assays

DiscoverRx Orphan GPCR Biosensor Cell Lines

DiscoverRx Class A Orphan Panel

Pairing of oGPCRs with Adopted Ligands

The following PathHunter oGPCRs have been paired with their adopted ligands. Against stimulation with the adopted ligands results in binding of arrestin to the ProLink tagged GPCR and an increase in enzyme activity. These results demonstrate the functionality of the assay with recently de-orphanized receptors.

oGPCR profiling with a Focused Compound Set

Panel:
48 of the PathHunter GPCR cell pools were screened against a panel of 275 GPCR-focused compounds. The cells were screened in 384-well dishes with a library concentration of 10μM. Example screening data is shown below.

Conclusions
• Arrestin binding occurs in the majority of GPCRs tested making the PathHunter Arrestin platform an ideal solution for de-orphanization programs.
• Interaction of the Arrestin-β Arrestin with the ProLink tagged GPCR is required for signal generation yielding a highly specific assay with fewer false positives than second messenger assays.
• DiscoverRx offers a panel of over 82 characterized oGPCR cell lines for research and drug discovery.
• A small scale screen yielded 4 confirmed hits on 3 different GPCRs, Validating PathHunter Arrestin Assays as valid approaches for GPCR de-orphanization.

Technology Access

Cell Lines
Finished cell lines containing the Arrestin-βA and oGPCR-ProLink fusions as heterogeneous pools, or cloned cell lines. ~80 available

Test Box
A clonal PathHunter parental cell line and a ProLink vector are available for build-your-own cell lines (7 cell backgrounds available)

Kits
Cells, detection reagents, and plates ready to go right out of the freezer. Each kit contains enough cells for 2 x 96 well plates

Discovery Partnerships
Custom screening and profiling against PathHunter oGPCRs