High-Throughput CellSensor® Reporter Assays For Multi-Pathway Analysis

Michael Hancock, Jun Wang, Jennifer Wilkinson, Thomas Machleidt, and Kun Bi
Invitrogen Corporation • Discovery Sciences • 501 Charmanny Drive, Madison, WI 53719 • USA

Abstract

Recent studies of cell signaling pathways revealed many genetic changes that contribute to various diseases. We have developed a portfolio of cell-based reporter assays that can be used to screen for perturbagens of over 20 different endogenous signaling pathways as well as specific protein targets (e.g. kinases) that are involved in these pathways. To build these multi-pathway reporter assays, we stably transduced the beta-lactamase reporter under the control of pathway-specific response elements as well as various cell backgrounds that natively express either wild-type or mutated signaling pathways. Activation of a pathway either through ligand binding or a clinically relevant constitutively active mutation in the pathway leads to the expression of beta-lactamase, which can be easily detected and quantified by a live cell, ratiometric beta-lactamase substrate. These cell lines were functionally validated with small molecule inhibitors and/or RNAi against known players of each pathway and were shown to be robust enough for high throughput screening. We have profiled 45 commercially available small molecule inhibitors against 11 signaling pathways. The results highlight the utility of multi-pathway cell-based profiling to advance drug discovery by providing robust data for both on and off-pathway activities.

Figure 1 - GeneBLazer® Beta-Lactamase Reporter Assay Technology

GeneBLazer® Technology uses a FRET-based substrate to provide reliable and sensitive detection of beta-lactamase activity. The advantages of this technology include:
1. Ratiometric readouts minimizes experimental noise.
2. Fluorescent readout allows for high throughput screening by flow cytometry.
3. Sensitive detection due to enzyme turnover of substrate.

Figure 2 - Invitrogen CellSensor® Cell Lines Offer Broad Pathway Coverage

Invitrogen Corporation • 1600 Faraday Avenue • Carlsbad, California 92008 USA • Telephone: 760 603 7200 • FAX: 760 602 6500 • Toll Free Telephone: 800 955 6288 • E-mail: tech_service@invitrogen.com • www.invitrogen.com