The use of novel endogenous reporter cell lines in high-throughput screening

Kam Dhaliwal 1, Holly Astley 1, Amy Hsu 2, Ruili Huang 2, Menghang Xia 2, Josh Bullock 1, Suzy Grooby 1, Mei Cong 3, Matt Robers 3, Kyla Grimshaw 1.

Horizon Discovery Ltd, Cambridge, UK  NCATS, NIH, Bethesda, Maryland, USA  Promega Corporation, Madison, Wisconsin, USA

Introduction

Reporter gene assays are widely used to study the regulation of gene expression. We have developed a suite of endogenous reporter cell lines which measure natural levels of protein expression and promoter activity. By measuring at the endogenous level, this system provides an advantage over other technologies which use exogenous plasmid-based overexpression systems.

Here we describe use of the HIF1A NanoLuc® protein reporter cell line to perform a high-throughput screen in 1536-well plate format using the NCGC Pharmaceutical Collection (NPC) of approved and investigational drugs.

Methods

Using adenovirus-associated virus based gene editing, one of the three technologies, alongside CRISPR, that comprise Horizon’s gene editing platform, we have engineered the endogenous HIF1A locus to generate an in-frame NanoLuc® luciferase protein fusion in the HCT116 colorectal cancer cell background. NanoLuc® luciferase is a small enzyme engineered for optimal performance as a luminescent reporter. The bright nature of NanoLuc® luciferase enables activity to be detected at endogenous expression levels.

Validation Results

Validation experiments were first performed to assess the utility of the HIF1A NanoLuc® protein reporter in drug discovery and development processes. Protein turnover was detected by using cycloheximide and protein accumulation was measured using bortezomib to block protein degradation. Furthermore, it was demonstrated that a linear relationship between luciferase signal and cell number is maintained even at low cell numbers, and decay kinetics showed a stable signal half-life.

Figure 2. Validation of the HIF1A NanoLuc® protein reporter cell line. (A) Protein turnover; (B) protein accumulation; (C) signal linearity; (D) stable decay kinetics. Assays were multiplexed with CellTiter-Blue® cell viability assay ensuring effects were not due to changes in viability.

Exposure to hypoxia (1% oxygen) for 4 hours resulted in the stabilisation of HIF1A protein leading to an increase in luciferase signal. Treatment with the HIF1A pathway inhibitor YC-1 decreased expression of HIF1A under hypoxia, as determined by NanoLuc® protein reporter.

These results were confirmed by Western blotting for HIF1A protein.

Validation Results

Cells were seeded into 1536-well plates and induction of HIF1A performed using 1% oxygen or the hypoxic-mimetic CoCl2. Knockdown of HIF1A protein reporter signal was confirmed using Topotecan. A high-throughput screen was run in 1536-well plate format using the NCGC Pharmaceutical Collection of approved and investigational drugs. The assay performed well with Z’ of 0.70 and CV of 6.1%.

High-Throughput Screen (HTS) Results

Initial results following a 16 hour drug exposure identified known regulators of the HIF1A pathway, highlighting the utility of this system for high-throughput screening.

Figure 5. Example data from HTS screen against 2014 NPC drugs in a 1536-well assay format.

Conclusions

We have generated novel protein reporter cell lines by introducing NanoLuc®, allowing us to study the modulation of endogenous protein stability in the high-throughput setting.

This technology could be applied to virtually any gene of interest and in a range of cell backgrounds, with resultant cell lines having applications in basic research as well as in high-throughput compound library screens.

For more information please visit  Booth #1528  for Horizon Discovery  and Booth #319 for Promega Corporation.  Further information on X-MAN® cell lines, technologies, available cell lines etc. is available at www.horizondiscovery.com.

Figure 2. Validation of the HIF1A NanoLuc® protein reporter cell line. (A) Protein turnover; (B) protein accumulation; (C) signal linearity; (D) stable decay kinetics. Assays were multiplexed with CellTiter-Blue® cell viability assay ensuring effects were not due to changes in viability.

Exposure to hypoxia (1% oxygen) for 4 hours resulted in the stabilisation of HIF1A protein leading to an increase in luciferase signal. Treatment with the HIF1A pathway inhibitor YC-1 decreased expression of HIF1A under hypoxia, as determined by NanoLuc® protein reporter.

These results were confirmed by Western blotting for HIF1A protein.

Validation Results

Cells were seeded into 1536-well plates and induction of HIF1A performed using 1% oxygen or the hypoxic-mimetic CoCl2. Knockdown of HIF1A protein reporter signal was confirmed using Topotecan. A high-throughput screen was run in 1536-well plate format using the NCGC Pharmaceutical Collection of approved and investigational drugs. The assay performed well with Z’ of 0.70 and CV of 6.1%.

High-Throughput Screen (HTS) Results

Initial results following a 16 hour drug exposure identified known regulators of the HIF1A pathway, highlighting the utility of this system for high-throughput screening.

Figure 5. Example data from HTS screen against 2014 NPC drugs in a 1536-well assay format.

Conclusions

We have generated novel protein reporter cell lines by introducing NanoLuc®, allowing us to study the modulation of endogenous protein stability in the high-throughput setting.

This technology could be applied to virtually any gene of interest and in a range of cell backgrounds, with resultant cell lines having applications in basic research as well as in high-throughput compound library screens.

For more information please visit  Booth #1528  for Horizon Discovery  and Booth #319 for Promega Corporation.  Further information on X-MAN® cell lines, technologies, available cell lines etc. is available at www.horizondiscovery.com.