APPLICATION OF BIOLUMINESCENCE AND CELL-BASED ASSAYS IN HIGH-THROUGHPUT SCREENING STUDIES

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Here we highlight several peer-reviewed publications citing use of Promega reagents and technologies in novel high-throughput screening applications.

Introduction

In the past decade high-throughput screening (HTS) has been used predominantly by the pharmaceutical and biotechnology industry for drug discovery. That landscape has changed somewhat in recent years. A variety of screening centers have been established in universities, government institutions and hospitals with the objectives of identifying not only potential drug leads, but also chemical probes that can be accessed by the public. Furthermore, HTS has also expanded to emerging markets where unique collections of compounds or extracts are used.

Here we highlight four peer-reviewed publications that cite the use of Promega reagents, reporters and bioluminescent assays. The paper by Lynch *et al.* illustrates the utility of the luciferase reporter assay for asking a specific question in a screening assay, rather than looking for general effects such as cytotoxicity. The article by Rossi *et al.* describes the application of a bioluminescent screening assay using low numbers of primary cells. Junker and Clardy have developed a bioluminescent HTS assay to identify inhibitors of bacterial biofilms. The research by Severson *et al.* describes the validation of a bioluminescent cell viability assay to identify compounds that inhibit the cytopathic effect (CPE) of SARS-CoV, an emerging infectious pathogen that requires BSL3 containment.

Lynch, R.A. *et al.* (2007) A small-molecule enhancer of signal transducer and activator of transcription 1 transcriptional activity accentuates the anitproliferative effects of IFN-γ in human cancer cells. *Cancer Res.* **67**, 1254–61.

STAT1 is a transcription factor that is involved in a variety of cellular processes and behaves like a tumor suppressor in many ways. It is associated with apoptosis and inhibition of cyclin-dependent kinases, and it may mediate the antitumor effects of IFN-γ and inhibit angiogenesis. The authors of this study designed a bioluminescent reporter assay to identify small molecules that enhance STAT1-dependent gene expression.

NIH3T3 cell lines were stably transfected with the firefly luciferase gene under the control of the STAT1 (STAT1luc/3T3) promoter. The control cell lines included a cell line stably transfected with firefly luciferase under the control of an NFκB promoter (NFκBluc/3T3) and a line stably transfected with firefly luciferase under the control of a constitutively active CMV promoter. Cells were plated at 4,000 cells/well in a 384-well plate, and 5,120 compounds from the Peakdale Library were added to the cells by pin transfer. Cells and compounds were incubated for 1 hour before adding IFN-γ. After a second incubation luciferase activity was assessed using the Bright-Glo™ Luciferase Assay System.

The bioluminescent reporter assay proved to be a robust assay with the STAT1luc/3T3 system giving a Z´-factor value of 0.92. The assay identified three compounds that increased STAT1-dependent luciferase activity by 1.5-fold. This increase in luciferase activity was not observed in the NFκBluc/3T3 system. Of the three compounds, 2-NP, was investigated further. 2-NP alone did not induce luciferase activity, but in the presence of IFN-γ, 2-NP increased luciferase activity 2.5 fold over the maximal induction seen with IFN-γ alone. The authors conclude that the major effect of 2-NP is to mediate the STAT1-dependent transcriptional events associated with IFN-γ induction.

To determine if the 2-NP effect was cell type-specific, a second Dual-Luciferase® Reporter screening assay was used. MCF-7 and SK-N-MC neuroblastoma cells were transiently transfected with the STAT1luc reporter construct and a plasmid containing *Renilla* luciferase under the control of the thymidine kinase promoter. The experiments indicate that the effect of 2-NP is not restricted to species or cell type.

The bioluminescent reporter assay used in this high-throughput assay worked with stably and transiently transfected cells. Additionally, because compounds that increased luciferase activity in this assay by default were cell permeant or able to reach their target without chemical assistance, the assay design excluded compounds that exhibited nonspecific toxicity.

Rossi, *et al.* (2007) Identifying druglike inhibitors of myelin-reactive T cells by phenotypic high-throughput screening of a small-molecule library. *J. Biomol. Screen.* **12**, 481–9.

Inflammatory T-cells are thought to be an important contributor in the pathogenesis of diseases such as Multiple Sclerosis and other diseases in which the immune system attacks myelin proteins within the central nervous system.

The authors isolated primary spleen cells from transgenic mice that express a T-cell receptor (TCR) that recognizes myelin proteolipid protein139-151 (PLP139-151) and use the CellTiter-Glo® Assay to screen for druglike compounds that inhibit proliferation of these T-cells in response to myelin PLP139-151. Compounds from the Laboratory for Drug Discovery in Neurodegeneration Library, which had been selected for oral bioavailability and blood-brain barrier penetration, were spotted onto 384-well plates and diluted. The diluted compounds were transferred to 384-well assay plates and spleen cell suspension from the transgenic mice was added. The spleen cell suspension contained medium only or medium plus PLP139-151. The cell suspensions were incubated and then proliferation was assayed relative to negative control cells (medium + vehicle; no PLP139-151) and positive control cells (PLP139-151 in medium + vehicle). Z´-factors for the HTS assay were robust (> 0.5), and the of the 41,184 compounds screened, 302 "hits" were obtained. The authors tested these 302 compounds for nonspecific toxicity in Jurkat cells using the CytoTox-ONE® Assay. Sixty three compounds were eliminated from consideration because of nonspecific effects. The remaining 239 compounds were evaluated further.

Junker, L.M. and Clardy, J. (2007) High-throughput screens for small-molecule inhibitors of *Pseudomonas aeruginosa* biofilm development. *Antimicrobial Agents Chemotherapy*. **51**, 3582–90.

Pseudomonas aeruginosa is an opportunistic pathogen that can form biofilms and establish persistent infections in compromised patients. Not only are these infections associated with Cystic Fibrosis and severe burns, but also surfaces such as catheters, ventilators, artificial joints and heart valves can promote biofilm formation. The cells of the biofilms are extremely slow growing and embedded in a polymeric matrix, making them resistant to antibiotics, host immune responses and other environmental stresses.

Psuedomonas aeruginosa cells are among the most difficult bacterial cells to lyse. This difficulty is compounded by the formation of biofilms. The authors of this study were able to acheive lysis-extraction-measurement in one step in a high-throughput screen to identify inhibitors of biofilm formation using the BacTiter-Glo™ Assay. They show that the BacTiter-Glo™ Assay produces higher Z´-values, is less messy, and provides reproducible results over a much greater range of bacterial OD than the standard crystal violet assay.

The authors screened over 66,000 compounds in 384-well plates. After plating the cells at 1×10^5 CFU/ml, test compounds were added, and the pin transfer tools were inserted into the wells of the growth plates to act as substrates for biofilm formation. After 24 hours, the pins were gently rinsed, transferred to an assay plate containing the BacTiter-Glo™ Reagent, and luminescence was measured as an indication of the bacterial cells that had attached to the pin tools. A detachment assay using a similar strategy was used to further characterize the "hits" from the primary screen. Thirty compounds representing six structural classes were identified as potent inhibitors of biofilm formation.

Severson, W.E. *et al*. (2007) Development and validation of a high-throughput screen for inhibitors of SARS CoV and its application in screening of a 100,000-compound library. *J. Biomol. Screen.* **12**, 33–40.

The authors of this paper describe the validation of a bioluminescent assay to identify novel compounds that inhibit the cytopathic effect (CPE) of SARS-CoV. SARS-CoV has proven refractory to standard viral treatments and has a mortality rate of 15%. Because it is a zoonotic virus, further epidemics of SARS-CoV are not predictable, and identifying effective treatment agents is essential.

Research on SARS-CoV must be conducted in a BSL3 containment laboratory. Any HTS assay to identify effective therapeutics against the virus must be easy to use and require minimal handling. In this study, three cell viability assay reagents were evaluated: MTS, Neutral Red and the bioluminescent CellTiter-Glo® Reagent. The CellTiter-Glo® reagent had several advantages over the other reagents including no requirement for washing or removal, minimal pipetting steps, and short incubation time, which reduced the time spent in the BSL3 laboratory.

The assay procedure involved plating cells at 10,000 cells/well in 96-well plates, incubating them for 24 hours, adding test compounds and then infecting the cells in the BSL3 laboratory with the Toronto-2 strain of SARS-CoV. The CellTiter-Glo® Reagent was added 72 hours after infection, and luminescence was read to assess cell viability.

This assay was validated against two small compound libraries, the Microsources Spectrum library and the Prestwick library. Compounds were screened at a single concentration for the primary screen. The assay produced Z´-factor values >0.6, signal-to-background values >16 and signal-to-noise values >3. Finally, the validated assay was used to screen 100,000 compounds from the National Institute of Neurological Disorders and Stroke library. The primary screen identified several compounds that inhibited CPE with a minimal effect on cell viability. Three of those compounds, from three distinct chemical classes, were characterized further as "highly active" and described by the authors as attractive lead compounds.

Summary

These four papers illustrate the potential of bioluminescent screening technologies to answer difficult questions in biology. Bioluminescence is ultra sensitive, allowing researchers to

assay small numbers of cells including primary cells or cells in mixed populations. Additionally, bioluminescence eliminates much of the background associated with test compound interference in screening assays, and since luciferase is not native to mammalian cells, researchers can design assays to ask very specific questions in a high-throughput setting.

For researchers interested in seeing other applications of bioluminescent technologies to the high-throughput arena, Promega has a list of some recently published peer-reviewed papers that have used cell-based assays and bioluminescent technologies to best advantage for high-throughput studies. This citation database can be accessed at: **www.promega.com/citations**. Just check the box for "high-throughput screening" on the search page and click "submit".

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Human Proteome Organization-US

Bethesda, MD, USA
16–18 March 2008
www.hupo.org

Japan Society for Bioscience, Biotechnology and Agrochemistry

Nagoya, Japan
26–29 March 2008
http://www.jsbba.or.jp/e/e_00/am_e.html

American Association for Cancer Research

SanDiego, CA
12–16 April 2008
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