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Overcoming technical challenges in cell-based high throughput screens

Tremendous technological advancements in automated biotechnology and combinatorial chemistry have led to the widespread implementation of high throughput screening (HTS) for drug discovery since the early 1990s. However, the drug discovery process is a long and costly journey, often requiring many years and millions of dollars to reach the market.

Moreover, while HTS may identify a couple of hundred lead compounds from a panel of hundreds of thousands or even millions of compounds, only a small fraction of these will ever be tested in a clinical trial, and an even smaller number will ever be approved by the Food and Drug Administration (FDA). Despite these difficulties, HTS remains a viable, but cumbersome, strategy for success.

Therefore, there has been a recent emphasis in the design and development of increasingly efficient and reliable HTS platforms for early phase drug discovery. In this whitepaper, we will:

- Outline some of the historical and contemporary strategies of cell-based HTS.
- Describe some of the technical challenges to overcome in successful HTS methods.
- Discuss some of the critical statistical concerns in HTS platforms, including common problems with false positives and strategies to increase statistical yield in cell-based HTS.

**Introduction to HTS platforms**

The two major strategies for HTS include biochemical and cell-based approaches. Pharmaceutical mediated biochemical approaches are typically target-based, utilizing enzyme inhibition or ligand-receptor interactions. However, as these biochemical HTS platforms are dependent on purified proteins, there are significant limiting issues related to protein stability and scalability. Furthermore, these approaches require subsequent analysis to test in-vivo or tissue-specific effects that cannot be obtained from initial biochemical screens.
Thus, the HTS field has continued to shift toward the development and utilization of in-vitro cell-based assays as a means for obtaining biologically relevant hits while simultaneously evaluating toxicity profiles early in the drug discovery process. By doing this, researchers aim to increase the success rate of the cell-based HTS approach by emphasizing ADMET (absorption, distribution, metabolism, excretion and toxicity) analysis of lead compounds earlier in the drug development process.

While the gold standard for this approach historically was animal models as proxies for humans, this has fallen out of practice for a number of logistical and ethical reasons. Therefore, it was in this context that the use of cell-based assays have become the mainstay for compound evaluation to obtain cytotoxicity, stability, permeability and growth effects of tested compounds in a more biologically relevant cellular environment.

Cell-based HTS has become increasingly popular as technological approaches have continued to become more sensitive, robust and reliable. Indeed, HTS laboratories perform more cell-based assays each year on average than the year prior, and in the past decade, cell-based assays now comprise more than 50 percent of all screening in HTS laboratories worldwide. Cell-based HTS platforms have been utilized throughout the early drug discovery process, from target or compound identification, to primary screening, lead identification and optimization, as well as safety and pharmacology/toxicology.

The three major types of cell-based HTS assays for compound screening include reporter gene, second messenger and cell proliferation assays. In particular, reporter gene assays are perhaps the most common, monitoring the expression of a reporter gene with a luminescent, colorimetric or fluorescent output monitored as a read-out of gene expression or cellular response.

Importantly, with increasing and widespread use of these platforms, the global market for cell-based HTS platforms in drug discovery for validating target compounds was estimated at $6.2 billion in 2010 with an estimated 11.6 percent projected compound annual growth rate. Moreover, the market is predicted to reach nearly $10.8 billion in
2015. Even the ADMET sector of drug compound testing was valued at $1.5 billion in 2010 and expected to nearly double in value to $3 billion by 2015 4.

These findings underscore the tremendous financial impact of cell-based HTS and the continued efforts to improve the biotechnology of this drug development process. However, despite the growing use and rise in popularity of cell-based HTS platforms, there remain significant technical and statistical challenges to overcome in optimizing this approach as discussed in detail below.

**Technical challenges of cell-based HTS**

There are significant technical challenges encountered in HTS that are unique to cell-based approaches as compared to more simplified biochemical assays. In particular is the use of cell lines that stably express a protein of interest, as this method is inherently difficult because it is a challenge to control expression levels after multiple passages.

Moreover, certain types of proteins can be more problematic than others to maintain adequate and reliable expression levels, including ion channels, certain GPCRs and proteins involved in cell death pathways that are maintained at low levels naturally. Some researchers combat this by freezing down large batches of cells to try and maintain consistent expression throughout the duration of the HTS run. However, there can be additional challenges in trying to maintain reliable expression with regard to growth conditions.

This is where having co-existing reference signals for cell viability that can identify toxicity conditions early on can be of benefit. In addition, even when these former issues are optimized, signal to noise can be problematic when using 1536 well formats, as the limited cells per well can make it difficult to reach robust signal to noise ratios over the threshold of reliable detection 5.

Importantly, there are numerous current strategies to overcome these barriers, including transient expression-based systems such as luciferase, fluorescent or viral
vector-based methods, as well as novel 3D culture, electrochemical and microfluidic methods, summarized elsewhere 1.

False hit rates and other statistical considerations in cell-based HTS

One critical aspect of cell-based HTS is the generation of multiple lead compounds that often result in a lot of wasted time, energy, and money. An even bigger problem is a false negative result which may lead to potential hits being overlooked due to lack of sensitivity in assay method of choice. The biggest contribution to the false hit rate has been the inability to use primary cells and assay for expression of gene activity in an endogenous environment. This has often led scientists to go back and reevaluate hits in an attempt to discern whether these compounds were successfully identified but not fruitful in the long run (i.e. excess toxicity or poor pharmacokinetics) or whether they were truly a false positive in the screening step in question.

Determining the true success rate of the HTS can help optimize future attempts and how much effort we should invest in compounds that did not make it to market. This brings into discussion some very important concepts in regard to screening tests. Namely, it highlights the some crucial concepts of testing that can help shape our understanding of how to optimize HTS assays.

If we look at these concepts in mathematical principles, then the sensitivity of a test (the proportion of positives that are correctly identified as such) would be defined as TP/(TP+FN), where TP is true positives and FN is false negatives. Conversely, specificity (proportion of negatives that are correctly identified as such) would be TN/(TN+FP), where TN is true negatives and FP is false positives.

Thus, the ideal screening test would be highly sensitive to thereby identify all possible hits, while subsequent confirmatory tests would ideally be highly specific, and therefore confirmatory. However, the strength of a test is also dependent upon the population being tested; namely, the importance of prevalence (in this case, the rate of potential positive compounds in the library being screened).
This is a critical concept, as increasing the prevalence will increase the pre-test probability and the positive predictive value of your test (thereby decreasing false positives).

Practically, there are various methods that could be employed for optimizing positive predictive value and decreasing false positives in cell-based HTS. One strategy would be to perform a virtual screen prior to a cell-based screen. This would require devising an algorithm to predict the hit rate in a given library using a parent molecule and/or its derivatives. By designing a method to perform a pre-test virtual screen, this could reduce the size of the potential compound screen, which would work to both make efficient use of resources as well as increase the pre-test probability and reduce the rate of false positives in your HTS.

Another strategy in its early stages is the use of RT-qPCR screening strategies to widen the dynamic range of screening technology to the native state. This method utilizes biologically relevant data by eliminating the need for protein overexpression by monitoring subtle endogenous changes in a primary cell population versus an engineered cell line. This is made possible by the workflow options, inherent sensitivity and dynamic range of PCR methods.

This strategy additionally highlights where future cell-based HTS assays are heading, including the use of primary cells, which have historically proven exceptionally difficult to utilize, as well as the ability to more readily identify subgroups within populations for which certain drugs may be better suited, thereby shifting HTS toward more personalized drug discovery methods.