1 Introduction

Traditionally high throughput screening has been run using a single concentration for each compound. So called "single point HTS" is cost effective, allowing for rapid screening of large numbers of compounds. The tested concentration tends to be high ($10\mu M$ is commonly used, though in certain paradigms such as fragment screening, much higher concentrations may be employed [REF]). In these types of screens, response data is normalized to positive and negative control wells and then hits are identified based on thresholds computed from the sample responses. The simplest approach is to select compounds whose response is beyond 3 standard deviations (SD) of the mean response across the entire screen. This is a very simplistic approach, and ignores aspects such as structure clustering and costs of screening. Thus methods have been devised to make more efficient selections from single point screens [8, 4, 9, 11, 6, 5].

Hits selected from a primary HTS must be confirmed in secondary and orthogonal screens, and a challenge for hit selection methods is to ensure a high confirmation rate. This can be challenging when employing a single point HTS. Compounds that show an extreme response (say $< 3SD$) may actually be cytotoxic and thus show a similar response at lower concentrations. Alternatively, the compound may only be active at the tested concentration and now show any activity at lower concentrations.

An alternative to single point HTS is to run a primary screen in dose response. This approach is termed quantitative HTS (or qHTS) [3] and by definition provides much more information about the compounds behavior in the assay. Naturally this is a more expensive and resource intensive but advances in automation and miniatureization technologies have enabled qHTS screen on over 300,000 compounds (using 5 to 7 concentrations depending on the assay). The result of a qHTS screen is a concentration response curve for each compound, providing key parameters including efficacy and a potency. Furthermore the shape of the curve (such as steepness of the linear region and presence or absence of asymptotes) can provide information on compound behavior [7]. As a result, compound selection from qHTS screens can be more refined. Aspects such as toxicity and potency can be identified in a qHTS screen, but not in a single point screen. Indeed, if one only considers activity in the screen (and no external information such as chemical structure or prior reports of activity), compound selection from qHTS screens should lead to a more reliable selection of true active compounds. One can also argue that a qHTS approach reduces the expense of secondary screening by passing on fewer hits (which hopefully reconfirm).

Nonetheless, a qHTS approach represents a significant investment in resources and one may ask whether qHTS is justified for a given assay. While there are economics aspects to this question, we focus on a simpler, functional analysis. Specifically, the goal of this study is to explore the overlap between hits selected purely based on activity from qHTS and single point screens.

Given that it is not feasible to run a qHTS screen followed by a single point screen on the same set of compounds, we consider a simulation approach, whereby we take a qHTS screen and the simulate a single point screen by analysing responses at one of the tested concentrations.

Table 1: A summary of the qHTS datasets used in this study.

Name	Description	Num. Compounds	Reference
Delayed death	Identify smalll molecules that lead to the delayed death phenotype in *P. falciparum*	164,457	[10]
FOXM1	Identify small molecular inhibitors of FOXM1 that block DNA binding	53,457	[2]
Yeast-PLP		325,004	

2 Methods

We identified a selection of screens run previously at NCATS, ranging from XXX to XXX compounds. For all screens well data were normalized to positive and negative controls and then 4-parameter Hill curves were fit using a grid based algorithm [REF]. The curve fitting procedure provide us with the fit parameters including the log potency (LAC50) and efficacy. In addition, we obtain a heuristic curve classification (CCLASS) that categories curve quality. Here, quality encompasses the accuracy of the curve fit and the shape of the curve. Thus good quality curves are defined as those with well defined upper and lower asymptotes and full (i.e., $> 80\%$ efficacy). Compounds with missing asymtpotes or partial efficacy are deemed inconclusive and finally compounds with no concentration response behavior are termed inactive. The classification scheme employs numeric labels - for antagonist screens, these are -1.1, -1.2, -2.1, -2.2, -1.3, -1.4, -2.3, -2.4, 3, and 4. Somewhat arbitrarily, classes -1.1, -1.2, -2.1 and -2.2 are classified as good, class 4 as inactive and the remainder as inconclusive. While the classification is based on arbitrary thresholds, it is useful as a guide to identifying good quality curves that one expects to confirm in a secondary screen.

2.1 Datasets

NCATS has run more than XXX qHTS screens since 2004. We selected two classes of screening datasets. The first set consisted of a set of 1912 compounds screened in 11-point concentration response, across a variety of cancer cell lines. Given the small size of this compound set, the increased cost of a qHTS approach is not significant. However, the use of these assays allows us to explore cell line specific effects on hits identified.

We also considered a set of large primary screens ranging in size from XXX to XXX compounds. For most of these screens, compound selection is involves analysis of a single assay. The exception is the delayed death screen in which hits are identified based on activity in two parallel screens.

3 Results

3.1 Delayed death

We next considered a qHTS screen [REF] that assayed for a delayed death phenotype [1] in *P. falciparum*. In this assay, a compound is considered a hit if it is inactive in the 48 hour assay but active in the 96 hour assay. The screen tested XXX compound at different numbers of concentrations. For this study we selected the set of XXX compounds tested at 6 concentrations at both 48 hour and 96 hour. Both assays were designed as loss of signal, such that smaller responses corresponded to more activity. As before, we identified the set of *true actives* based on the qHTS results. Specifically, compounds that were class 4 with a median response ¿ 80 in the 48 hour screen and were class -1.1, -1.2, -2.1, -2.2 in the 96 hour screen with efficacy > 75 were selected. This resulted in XXX compounds.

We then considered the single point version. For a given concentration, C_i, responses were converted to robust Z-scores [REF]. We then selected a Z threshold, Z_j such compounds with response $> Z_j$ in the 48 hour screen and respone $< Z_j$ in the 96 hour screen were selected as hits. We considered a sequence of Z thresholds for each concentration.

As before, if we consider the two assays independently, we observe a monotonic increase in the fraction of true actives identified from the single point screen with increasing Z-score threshold. It is interesting to note that the the second highest concentration identifies more true actives at every Z-score threshold compared to the top concentration. As noted above, this is likely due to toxicity effects occuring at the top concentration.

In contrast, when we consider the more complex selection rule to identify hits exhibiting the delayed death phenotype, we observe a different trend. In this case, after a certain Z-score threshold the number of true actives identified by the single point screen drops to zero. In contrast to threshold based selection for one screen, the delayed death screen involves two assays and thus the selection is defined by an area described by the two thresholds. Figure **??** plots the activity of each compound in the 48 hour and 96 hour screens, overlayed with the $Z = 3$ tresholds. It is apparent that the selection region (lower right quadrant) is populated with compounds. Yet none of these overlap with the qHTS derived hits.

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