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SPECIAL REPORT

Yeast: bridging the gap between phenotypic and biochemical assays for high-throughput screening

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ABSTRACT

Introduction: Both *in vitro* biochemical and phenotypic assay platforms have clear limitations in high throughput screening (HTS) for drug discovery. The use of genetically tractable model yeast as a vehicle for target-based HTS overcomes many of these by allowing the identification of on-target compounds that function within a eukaryotic cellular context.

Areas covered: In this special report, the use of yeast-based assays in HTS is discussed with reference to the various platforms that have been utilized over the past 20 years. The specific issues considered are the necessity to employ counter and secondary screening approaches to ensure the on-target activity of hits, and the recent developments in detection systems that have facilitated miniaturization and ultra-HTS.

Expert opinion: It is difficult at present to predict the future. That being said, the demonstrable possibilities of optimizing yeast-based HTS, coupled with the demonstration of utility in an industrial setting, shows that these platforms have the potential to bridge the gap between phenotypic and biochemical assays for HTS.

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1. Introduction

High-throughput screening (HTS) facilitates the analyses of vast numbers of compounds (≥100,000/day) for biological activity, an approach central to modern drug discovery efforts. HTS assays formats can, in general, in divided into two categories: *in vitro* biochemical and cell-based, phenotypic assays. The former are often simpler and more specific, whereas cell-based phenotypic assays can be more challenging but provide cellular context [1].

For the automated HTS required to achieve true high content screening, all assays must be as simple as possible. For example, *in vitro* biochemical assays must produce a readily detectable and quantifiable signal (fluorescence, radiochemical, etc.) without complex substrate–product separation. This necessitates the isolation of pure, soluble, and enzymatically active protein; clearly these requirements can be major obstacles [2,3]. In contrast, cell-based phenotypic HTS platforms do not require target protein purification nor any knowledge of the target or targets, and compound activity is established in a cellular context that mirrors the *in vivo* scenario, for example, the necessity for a compound to cross membrane barriers to reach an intracellular target, thus predicting those compounds least likely to exhibit problems with stability or solubility *in vivo* and fast-tracking drug-like entities for further development. However, these advantages can also be detrimental. For example, compounds that fail to cross membrane barriers and reach the target could represent promising early stage hits, and their loss precludes further development. In addition, the nontarget-based phenotypic readout requires that target deconvolution is approached *a posteriori* to facilitate rational development of compound hits [2,3].

In summary, both approaches have distinct challenges which are summarized in Table 1. However, to best reflect the physiological context in which a compound has an effect, cell-based assays have obvious advantages [4]. Engineering the cell to provide a target-specific readout is possible, thereby combining the advantages of both *in vitro* biochemical and cell-based, phenotypic assays. However, target eukaryotic cells can be difficult and expensive to culture and genetically manipulate [5], in addition the process redundancy found in complex systems can cofound the readout of HTS for a specific inhibitor [6]. Therefore, whilst cell-based systems clearly provide some significant advantages compared with conventional target-based *in vitro* approaches, their use is not without limitations.

Yeast, particularly *Saccharomyces cerevisiae*, but also *Schizosaccharomyces pombe*, have been long established as relatively simple, tractable, and highly characterized model eukaryotes [7]. Yeast benefit from a highly developed genetic tool kit and are fast growing (doubling time ~90 min) in simple, low-cost (liquid or solid) media. Such characteristics make *S. cerevisiae* and *S. pombe* ideal for antifungal HTS [8]. However, given that many cellular pathways are conserved in yeast and other eukaryotes (e.g. humans), these model cells can be easily engineered for the analyses of orthologous functions [9]. For example, the use of the yeast two hybrid systems has been utilized to identify inhibitors of protein–protein interactions

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Article highlights

- Yeast-based screening has the potential to *bridge the gap between phenotypic and biochemical assays for HTS*
- Lethal expression, transactivation and substitution yeast-based assays have all been effectively employed in HTS, including ultra HTS
- Exploiting the genetic tractability of yeast to overcome platform limitations is possible, e.g. deletion of efflux pumps and use of fluorescent markers for multiplexing
- Recent developments, including the identification of a clinical candidate for Parkinson's disease, have shown that yeast-based HTS can take its place amongst the currently utilized drug discovery platforms
- Whilst the future is difficult to predict, yeast-based platforms are well positioned to take their place in the armory of drug discovery techniques in coming years

This box summarizes key points contained in the article.

[10,11]. However, the application of this technology for drug discovery has been relatively limited. In the following sections, I will focus on the wider use of yeast-based platforms designed to identify inhibitors of protein function. The predominant platforms have been previously described and illustrated [2,3], and in the following sections the contribution of yeast to HTS and drug discovery will be further discussed with reference to the available literature (Table 2, in chronological order) and the future perspectives considered.

2. Yeast HTS platforms

2.1. Lethal expression

Given the highly developed genetic tool kit available to modify the yeast genome and express axenic proteins, both *S. cerevisiae* and *S. pombe* have been successfully engineered into reporter systems that have been utilized in HTS. For example, expression of the influenza virus ion-channel forming protein in *S. cerevisiae* is lethal [12]. The expression of pro-apoptotic HIV-1 protein Vpr is similarly lethal to the fission yeast *S. pombe* [13]. For both cases, this lethal phenotype has been utilized to identify molecules that rescue the yeast, i.e. inhibit orthologous protein function. Lethal expression has also been used in yeast-based screens for modulators of human proteins [4,6]. For example, both the human kinase p38α [14] and phosphatidylinositol 3-kinase (PI3K) [15] are associated with oncogenesis (and a plethora of other diseases, Table 2) and their expression is toxic to *S. cerevisae* [16,17], as is the expression of α-synuclein (α-syn) associated with Parkinson's disease [18–20]. Similarly, recent work described an *S. cerevisiae* lethal expression platform in which yeast deficient in uridine transport were made susceptible to toxic 5-FUrd by the expression of Nucleoside Transporter 1 from the malaria parasite *Plasmodium falciparum*. These were then utilized in HTS to identify antimalarial compounds [21]. Importantly, in all phenotypic rescue assays the possibility of generically toxic false positives is removed greatly simplifying the generation and interpretation of data.

2.2. Transactivation

Lethal expression-based platform readout is reliant on positive growth changes in response to the direct chemical modulation of the target protein. However, given the extreme genetic tractability of both *S. cerevisiae* and *S. pombe,* it is possible to engineer these model yeast to provide an indirect HTS readout through transactivation. Several examples have been reported [22,23] (Table 2), but the most extensively described and HTS utilized example of a yeast transactivation platform has been directed toward the discovery of mammalian phosphodiesterase (PDE) inhibitors [24–28]. The approximately 100, tissue specific, mammalian PDE isoforms all catalyze the conversion of cAMP and cGMP to the cyclic secondary messengers 5′AMP and 5′GMP. Given the tissue specificity of many of these isoforms, selective modulation has been recognized as having therapeutic potential for a wide range of human diseases [29,30] (Table 2). *S. pombe* can tolerate deletion of adenylate cyclase (*cyr1)* and PDE (*csg2*). In their absence, exogenous cAMP or cGMP activates Protein Kinase A (PKA) and *ura4* expression from an introduced selectable marker is repressed. In the absence of *ura4* expression, the yeast can grow in the presence of 5-fluoroorotic acid (5FOA), which is converted to toxic fluoroorotidine monophosphate in the presence of URA4 [28,31]. However, expression of yeast (Cyr1) or a mammalian PDE facilitated the conversion of cAMP or cGMP to 5′AMP or 5′GMP, leading to *ura4* expression and 5FOA sensitivity. Inhibition of PDE function rescued growth and the system has been formatted into a positive selection HTS

Table 1.

	Advantages	Disadvantages
***In vitro* HTS**	Possibility of simple, specific, and sensitive assay system Facilitates SAR based on molecular recognition of the target	Requires purified target, limiting assay of hard to purify/assay targets such as transmembrane enzymes Expensive due to necessity for protein purification, etc. Requirement for suitable substrates
Phenotypic HTS	Does not require purified target Provides specific cellular context allowing early selection of drug-like compounds Naïve approach challenging all targets at once in an unbiased manner	Relatively insensitive due to drug pumps and membrane barriers Expensive or inability to culture relevant cell type Reliance on non-target specific, phenotypic output SAR is not assisted by target knowledge. Target deconvolution is approached a posteriori
Yeast-based HTS	Does not require purified target Provides axenic eukaryotic cellular context allowing early selection of drug-like compounds Ease of manipulation and speed of growth Straightforward genetic manipulation for generic assay platforms Ease of discrimination of false positive hits Low cost of culture	Relatively insensitive due to drug pumps, membrane barriers, and the thick cell wall Often reliant on the ability of an heterologous protein to be functional in an axenic system Target protein is tested in a non-native cellular *milieu*

Table 2.

Organism	Disease/effect	Target(s)	Assay	Library/hits	Outcome	Reference
Influenza virus	Influenza	Ion-channel forming M_2	Lethal expression in *Saccharmoyces cerevisiae*, 96-well plates	*'Compounds drawn from the Bristol-Myers Squibb compound collection,'* 1 hit	*In vivo* activity in plaque assay (µM)	Kurtz et al. 1995 [12]
Haemonchus contortus	Anaemia and edema in ruminants	Orthithine decarboxylase	Substitution in *S. cerevisiae*, 96-well plates	*'A subset of a collection of synthetic compounds,'* (90,000), 1 hit	Single hit shown to be not on target *in vitro* and *in cellulo*	Klein et al. 1997 [35]
Human	Neurological trauma	Calcium channel (subunits α1B and β3)	Transactivation (2 hybrid) in *S. cerevisiae*, agar plates	Subset American Home Products Corporate (156,000), 10 hits	Specific *in cellulo* activity (µM)	Young et al. 1998 [49]
Human	Cancer, diabetes, cardiovascular diseases, neuro-degenerative diseases	Sirtuin (SIRT2)	Transactivation in *S. cerevisiae*, 96- and 384-well plates	IICB Diversity and Chem-Bridge (1600), 3 hits	Specific *in vitro, in cellulo* (mammalian) and *in vivo* (plant) activity (µM)	Grozinger et al. 2001[4] [22]
Human	Prion diseases	Prion protein (PrP)	Lethal expression in *S. cerevisiae*, agar plates	*'Synthetic and natural products purified from various sources by academic laboratories'* (2500), 6 hits	Specific *in cellulo* activity in yeast and mammalian systems (µM)	Bach et al. 2003 [46], 2006 [47]
Human	Alzheimer's disease	β-secretase	Transactivation in *S. cerevisiae*, 96-well plates	*'Chemical compound library'* (15,000), 13 hits	Specific *in cellulo* activity (µM)	Middendorp et al. 2004 [23]
Human	Inflammatory diseases, cancer, congestive heart disease	Protein kinase p38α	Lethal expression in *S. cerevisiae*, 96-well plates	ChemDiv combinatorial (40,000), 2 hits	On target *in vitro* and *in cellulo* activity (nM-µM)	Freidmann et al. 2006 [17]
Human	Parkinson's disease	α-synuclein (α-syn)	Lethal expression in *S. cerevisiae*, agar, 96- and 384-well plates	*'collection of 10,022 diverse compounds'* from ComGenex and Sigma Aldrich Expressed cyclic peptide library (5M) *'Over 115,000 compounds from various collections, including commercial libraries, natural products and National Cancer Institute collections'*	Active flavonoids identified Specific *in cellulo* activity in nematode model system (µM) Specific *in cellulo* activity in nematode and mammalian model systems (µM)	Griffioen et al. 2006 [18] Kritzer et al. 2009 [19] Su et al. 2010 [20]
Human	Neurological dis- eases, inflammatory diseases, metabolic diseases, memory loss, cancer, erectile dysfunction	Phosphodiesterase (PDE) 2A/4A/4B/8 PDE7 PDE11 PDE4/8 PDE 4/7	Transactivation in *S. pombe*, 384-well plates	*'Subset'* Prestwick Bioactive & Microsource Spectrum (3120), 151 hits *'Commercial chemical libraries,'* (48,176), 5 hits Including Actimol TimTec1, ChemBridge1,3, ChemDiv3&6 (198,382), 39 hits BIOMOL2 ICCB, Prestwick1, NINDS2, Asinex1, Biomol-TimTec1, Bionet1-2, CEREP, ChemBridge3, ChemDiv 1–4&6, Enamine1-2, I.F. Lab2, Life Chemicals1, MayBridge1-5, MixCommercial1-3&5, and Peakdale (222,711), 367 hits Broad Institute (undefined), 163–1610 hits	On target (PDE4B) *in cellulo* activity (µM) On target *in cellulo* and *in vitro* activity (µM) On target *in cellulo* and *in vitro* activity (µM) On target *in vitro* activity (nM) and elevation of cell testosterone and progesterone *in cellulo* (µM) On target *in vitro* activity (nM), anti-inflammatory action *in cellulo* (µM) and molecular docking (low energy)	Ivey et al. 2008 [28] Alaamery et al. 2010 [24] Cayhan et al. 2011 [25] Demirbas et al. 2013 [27] Santos de Medeiros et al. 2017 [26]
Human	Anderson sydrome	Potassium channel (Kir2.1)	Substitution in *S. cerevisiae*, 96-well plates	MicroSource Natural Products Library (720), 1 hit	On target *in cellulo* activity in yeast and mammalian cells (µM)	Zaks-Makhina et al. 2009 [32]
Human	Obesity, cancer	Acetyl-CoA carboxylase 1/2 (ACC1/2)	Substitution in *S. cerevisiae*, 96-well plates	ChemDiv: *'ACC1 Structure-Activity Relationship Library'* (3068); *'Diverse screening library'* (30,000); *'ACC2 Analog Screening Library'* (828), 38 hits	On target *in vitro* activity selective for AAC2 (µM)	Marjanovic et al. 2010 [33]
HIV-1	AIDS	Viral protein R	Lethal expression in *Saccharmoyces pombe*, 384-well and agar plates	Microsource Spectrum Collection (2000), 1 hit	Confirmation of anti-apoptotic effect (µM)	Benko et al. 2010 [13]
Human	Mitochondrial disease	Mitochondrial ATP synthase (ATP6)	Lethal expression in *S. cerevisiae*, agar plates	*'Compounds from various chemical libraries'* (12,000), 10 hits	On target *in cellulo* activity in mammalian cells (µM)	Couplan et al. 2011 [48]
Human	Cancer, autoimmune diseases, metabolic disorders, atherosclerosis cardiovascular disease	Phosphatidyl-inositol 3-kinase (PI3K)	Lethal expression in *Saccharmoyces cerevisiae*, 96-well plates	*'Collection of natural products of microbial origin'* (9600), 55 hits	On target *in cellulo* (µM)	Fernandez-Acero et al. 2012 [16]

(*Continued*)

Table 2. (Continued).

Organism	Disease/effect	Target(s)	Assay	Library/hits	Outcome	Reference
Plasmodium spp, *Schistosoma masoni, Trypanosoma* spp, *Leishmania major*	Malaria Schistosomiasis African/American trypanosomaisis Leishmaniasis	Dihydrofolate reductase (DHFR), N-myristoyl-transferase (NMT), Phosphoglycerate kinase (PGK)	Substitution in *S. cerevisiae*, multiplexed, 384-well plates	Maybridge Hitfinder (14,400), 445 hits	Anti-*T. brucei* activity *in cellulo* (nM)	Bilsland et al. 2013 [37]
Human	Cancer, diabetes, cardiovascular disease, obesity, male contraception	Adenine Nucleotide Translocase 4 (ANT4)	Substitution in *S. cerevisiae*, 384-well plates	65,000 compounds from '*commercial sources*,' 19 hits	On target activity *in vitro* (μM)	Zhang et al. 2016 [34]
P. falciparum	Malaria	Nucleoside Transporter 1	Substitution in *S. cerevisiae*, 384-well plates	'*several chemical libraries*' (64,560), 171 hits	On target cellular activity (nM) and anti-parasitic activity against infected erythrocytes (μM)	Frame et al. 2015 [21]
Brugia malayi	Lymphatic filariasis	NMT, PGK, triose-phosphate isomerase, adenosyl homocysteinase, thymidylate synthetase, ribose-5-phosphate isomerase, deoxyhypusine synthase, CDP-alcohol phosphatidyl transferase	Substitution in *S. cerevisiae*, multiplexed, 384-well plates	Malaria Box (400), 9 hits	Anti-*B. malayi* activity *in cellulo* (μM)	Bilsland et al. 2016 [36]
Leishmania spp	Leishmaniasis	Inositol phosphoryl-ceramide (IPC) synthase	Substitution in *S. cerevisiae*, 1536-well plates	GSK library (1.8M), 500 hits	On target *in vitro* and *in cellulo* activity (nM-μM) and anti-leishmanial activity *in cellulo* (nM)	Norcliffe et al. 2018 [41]
Plasmodium spp	Malaria	DHFR	Substitution in *S. cerevisiae*, multiplexed, 384-well plates.	Johns Hopkins FDA & foreign approved library (1600),1 hit	On target *in vitro* (nM), anti-*P. falciparum* activity *in cellulo* (μM) and molecular docking (low energy)	Bilsland et al. 2018 [38]

used to screen libraries >200k in size [24–28]. To ensure specificity against single or small numbers of PDE isoforms screening against yeast expressing different isoforms can be undertaken, so-called counter screening. Using this approach, many of the hits were demonstrated to be specific for one or more PDE isoforms [24–28]. Again, like the lethal expression platforms, these phenotypic rescue assays reduce the likelihood of false-positive hits.

2.3. Substitution

Despite the advantages (e.g. reducing false positives) of lethal expression and transactivation platforms, the majority of reported yeast-based HTS have relied on the simple substitution of an essential yeast function with the orthologue from the organism of interest. Of course, this is dependent of the conservation of protein function between the yeast and an often distantly related eukaryote. This approach has been widely used in HTS of mammalian targets [9]. For example, mutant *S. cerevisiae* complemented by expression of a human potassium channel protein (Kir2.1) was formatted into an HTS assay where inhibition at low but not high potassium was scored as a hit [32]. Similarly, with a view to discovering hit compounds toward the treatment of obesity, human acetyl-CoA carboxylase 2 (AAC2) inhibitors were identified by HTS using mutant *S. cerevisiae* reliant on expression of human AAC2 [33]. More recently, a *S. cerevisiae* substitution platform was employed for HTS to identify inhibitors of human adenine nucleotide translocase 4 (ANT4), a target for cancer and cardiovascular disease among others [34]. In both cases, counter screening against yeast complemented with isoforms of the target (human AAC1 and ANT1, −2 and 3, respectively) ensured selectivity [33,34].

However, the first report of the use of a substitution platform in HTS was against the ornithine decarboxylase (ODC), a key regulatory enzyme in eukaryotic polyamine (PA) biosynthesis, from *Haemonchus contortus*, a nematode worm that is a major parasite of ruminants. Similar to above, the platform utilized a *S. cerevisae* ODC deficient mutant complemented by expression of the *H. contortus* orthologue [35]. Subsequently, recent years have seen most published activity using *S. cerevisiae* substitution platforms directed to enable HTS against parasitic proteins [2,3]. For example, *S. cerevisiae* mutant yeast have been complemented by the expression of dihydrofolate reductase (DHFR), *N*-myristoyltransferase (NMT), phosphoglycerate kinase (PGK), and several other targets (Table 2) from protozoan and helminth parasites [36–38]. An *S. cerevisiae* substitution platform has also been used in HTS to identify inhibitors of the anti-leishmanial drug target inositol phosphorylceramide (IPC) synthase [39–41]. In this case, in the absence of a mammalian orthologue [42–45], counter screening was performed against the mutant complemented with the yeast orthologue AUR1 [41] to remove off-target, generically cytotoxic compounds.

3. Detection and sensitivity

Many of the initial studies utilized agar plate-based assays to create reporter systems [46–49]. However, it is clear that the measurement of growth on agar plates can only be semi-quantitative, even when colourimetric [46,47]. Therefore, the majority of yeast-based HTS now establish growth in liquid culture in microtiter plates, via optical density, colourimetry, fluorimetry, or a combination of these [21]. The great advantage is that this liquid format is amenable to miniaturization from 96-well to 384-well, and even to 1536-well [41] plates (Table 2). The vast majority of assays have used turbidity (measured via optical density) as a measure of liquid growth [2,3], sometimes with measures to enhance sensitivity such as deletion of efflux pumps, to reduce expulsion of potential inhibitory compounds, and non-lethal disruption of the plasma membrane [16]. However, a fluorescent readout provides considerably greater sensitivity [50] and has been recently employed in yeast-based HTS. For example, Bilsland et al. (2013) employed fluorescent tags to facilitate multiplexing, thus allowing compounds to be screened against protozoan DHFR, NMT, or PGK (Table 2) simultaneously with the human orthologue in a single well of a 384-well plate [37]. More recently, fluorescein di-β-D-glucopyranoside (FDGlu; a highly sensitive substrate for the glucosidases found in the yeast cell wall) was utilized to measure yeast growth in a HTS campaign against the *Leishmania* IPC synthase. The sensitivity of this approach allowed the assay to be miniaturized into 1536-well plates for screening against 1.8M compounds [41], to date the only published example of a yeast-based ultra-HTS.

4. Secondary assays

A significant advantage of the transactivation and lethal expression platforms described above is that they rely upon positive selection, thus reducing the number of generically toxic false-positive hits. In contrast, the negative selection employed in substitution assays necessitates counter screening in eliminate false positives. However, despite this clear advantage, all yeast-based platforms have the inherent possibility of producing false-positive hits, for example, due to inhibition of other elements within a biosynthetic pathway [2,3]. Therefore, as with all HTS, secondary screens to ensure that the identified hits are *on target* are an essential tool is the discovery process. These can be *in vitro* enzymatic and/or cell-based assays. For example, some hits identified in the multiple HTS (3k to >200k compounds) using PDE transactivation 384-well platforms, demonstrated selective PDE inhibition and physiological activity (e.g. cAMP elevation) in secondary assays [24–28]. Similarly, both of the two (from 40,000 compounds) p38α hits from the lethal expression assay demonstrated activity in *in vitro* and in mammalian cell secondary assays [17], and two cyclic peptide hits identified as active against α-syn in yeast prevented dopaminergic neuron loss in a *Caenorhabditis elegans* Parkinson's model [19].

In assays designed to identify anti-infectives, secondary assays often involve establishing activity against the pathogen. For example, of the specific hits identified by Bilsland et al. (2013) in the multiplexed fluorescent *S. cerevisiae* substitution platform, 50% proved cytotoxic to the protozoan parasite *Trypanosoma brucei in cellulo* [37]. However, this did not directly prove that the target was attenuated, in other work approaches involving *in vitro* and cell-based assays have

demonstrated that the anti-parasitic hits generated from yeast-based screens do actually hit the intended target [21,38,41].

The necessity of the secondary assay approach, target directed and across both *in vitro* and *in cellulo* platforms, was illustrated very clearly in one of the very first yeast-based HTS campaigns from 1997 [35]. Klein et al. identified a single, *selective*, compound following HTS and counter screening in conditions where the target, helminth ODC, is redundant. However, this hit failed to inhibit the target *in vitro* instead, perhaps, inhibiting a yeast enzyme involved in spermidine and spermine synthesis [35].

5. Conclusion

As discussed above, yeast-based screening methodologies offer considerable benefits for HTS toward drug discovery (Table 1). Using their genetic tool box, yeast-based assays that facilitate a target-directed approach within a eukaryotic cell environment can be engineered to *bridge the gap between phenotypic and biochemical assays for HTS*. Such yeast-based assays are robust (Z-factor ≥0.5 [13,16,26,31,41]) and cost effective – using simple 'mix and measure' approaches with output measured by monitoring cell growth in liquid media. Although most of those reported achieve this via optical density, more recent yeast-based HTS has employed fluorometric responses [21,36–38,41]. This has enabled miniaturization and high content screening using 384-well [21,36–38] and, recently, 1536-well [41] platforms.

Although yeast-based screens have limitations (Table 1), the capacity for ultra-HTS of millions of compounds against a target in a eukaryotic cellular context, when allied with necessary counter and secondary screens, makes yeast a promising tool for the discovery of *drug-like* hit compounds.

6. Expert opinion

The capacity to screen for specific target inhibition in a manipulable and well-controlled eukaryotic cellular context, means that yeast-based HTS combines many of the advantages of conventional *in vitro* biochemical and phenotypic screening approaches (Table 1). The use of a relatively simple eukaryotic *vehicle* also reduces many of the confounding effects caused by biological pathway redundancies in higher organisms. However, since the first reported yeast-based HTS in 1995 [12] less than 30 (many iterative) such campaigns have been published in the scientific literature (Table 2), a rate of little more than 1 per year. This relatively low level of visible activity is a limitation on the development of these platforms, which of course have challenges such as the presence of a thick cell wall and highly expressed efflux pumps which reduce assay sensitivity. Although genetic and chemical approaches to overcome these have been employed [16,51] their use, and so validation, is limited. Furthermore, the use of sensitive fluorescent approaches and miniaturization toward ultra-HTS is recent and only deployed for a limited number of targets [21,36–38,41].

This lack of evidential research explains why no record of lead to hit drug discovery programs stemming from yeast-based HTS could be identified in the literature. Although, importantly, Yumanity Therapeutics (co-founded by Susan Linquist [18–20]) have very recently (September 2018) issued a press release reporting the first clinical candidate (YTX-7739) for Parkinson's disease discovered through their proprietary integrated discovery platform which includes a yeast-based component. Furthermore, very recent work has demonstrated the tractability of these HTS systems for screening *hard to purify/assay* proteins (e.g. transmembrane enzymes involved in lipid biosynthesis) in a robust miniaturized (1536-well) platform against a 1.8 million compound library [41]. This demonstrated that yeast-based platforms have huge potential to be deployed in industrial drug discovery, for the reasons cited above coupled with the fact that these rapidly growing, simple eukaryotes are cheap and easy to cultivate and manipulate. To fully exploit the opportunities, yeast-based HTS provides a number of challenges need to be addressed. While 1536-well ultra-HTS has been reported [41], the fluorescent signal measured relied upon the addition of an expensive external agent (FDGlu) which required additional buffering [41]. The use of fluorescent proteins (either tagged target or expressed independently) has been reported and used to multiplex yeast-based HTS platforms [36–38]. This has great potential advantages; however, the differential fluorescence seen in cell population expressing these proteins from the transformed plasmid [37] would prove problematic in any effort to achieve ultra-HTS. Indeed, in our ultra-HTS efforts [41], we abandoned this approach (Mina and Denny, unpublished). The use of clonal, stable yeast cell lines with integrated fluorescent markers should overcome this limitation and open the door for multiplexed ultra-HTS which would allow the selection of target specific compounds in a relatively cheap and efficient manner.

Therefore, while the future is difficult to predict, yeast-based platforms are well positioned to take their place in the armory of drug discovery techniques in coming years, alongside conventional *in vitro* biochemical and phenotypic approaches, and fragment-based [52] and virtual screening [53,54]. While this may well happen out of sight of the academic community, such an increase in activity will increase the probability of compound hits entering hit to lead and preclinical trials programs.

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Declaration of interest

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