FEMS Yeast Research, 2025, **25**, foaf013
DOI: 10.1093/femsyr/foaf013
Advance access publication date: 20 March 2025
Minireview – Genetics, cell biology & metabolism

Advances in CRISPR-enabled genome-wide screens in yeast

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Editor: [John Morrissey]

Abstract

Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas genome-wide screens are powerful tools for unraveling genotype–phenotype relationships, enabling precise manipulation of genes to study and engineer industrially useful traits. Traditional genetic methods, such as random mutagenesis or RNA interference, often lack the specificity and scalability required for large-scale functional genomic screens. CRISPR systems overcome these limitations by offering precision gene targeting and manipulation, allowing for high-throughput investigations into gene function and interactions. Recent work has shown that CRISPR genome editing is widely adaptable to several yeast species, many of which have natural traits suited for industrial biotechnology. In this review, we discuss recent advances in yeast functional genomics, emphasizing advancements made with CRISPR tools. We discuss how the development and optimization of CRISPR genome-wide screens have enabled a host-first approach to metabolic engineering, which takes advantage of the natural traits of nonconventional yeast—fast growth rates, high stress tolerance, and novel metabolism—to create new production hosts. Lastly, we discuss future directions, including automation and biosensor-driven screens, to enhance high-throughput CRISPR-enabled yeast engineering.

Keywords: functional genetics; forward genetic screening; nonconventional yeast; CRISPR; metabolic engineering

Introduction

Functional genomic screens allow the discovery of genotype–phenotype relationships and interactions between genes (Schuldiner et al. 2005, Suter et al. 2006, Holland and Blazeck 2022). To do this, gene expression is eliminated or modulated and cells are screened to identify any resulting phenotypic changes (Doench 2018). Before whole-genome sequencing and the advent of clustered regularly interspaced short palindromic repeats (CRISPR)-Cas technologies, these screens were typically performed with random UV (Pringle 1975) or chemical mutagenesis (Kilbey 1975). Now, manually generated knockout libraries (Winzeler et al. 1999, Baba et al. 2006), RNA interference (RNAi) (Boutros and Ahringer 2008), transposons (Michel et al. 2017), or guide RNAs (gRNAs) as part of a CRISPR-Cas system (Robertson et al. 2024a) can be carefully designed to specifically target all or a subset of genes in a host organism. In yeast, CRISPR-Cas genome-wide screens can be easily designed to target all genes (Ramesh et al. 2023, Tafrishi et al. 2024), or guides can be multiplexed to study genetic interactions (Schuldiner et al. 2005, Costanzo et al. 2019). After altering the genotype of a pool of mutants, further assays can be performed to identify genes of interest. For example, survival assays can determine gene essentiality (Suter et al. 2006), genes needed for fitness in stress conditions (Robertson et al. 2024a), or for the production of metabolites (D'oelsnitz et al. 2022, Robertson et al. 2024b).

The use of functional genomic screens in yeast has proven their ability to elucidate gene function and gene interactions, which allows for knowledge generation about the genetic underpinnings of biotechnology traits. In addition to this, functional genomic screens are inherently powerful for engineering microorganisms by performing screens in stress conditions (Ando et al. 2006, Teixeira et al. 2010, Ramesh et al. 2023), on low-value feedstocks (Usher et al. 2011, Coradetti et al. 2018, Robertson et al. 2024a), or for improvement in metabolite production (D'oelsnitz et al. 2022, Liu et al. 2022a). Yeast hosts for industrial production of chemicals and bioproducts benefit from high tolerance to environmental stresses and broad substrate metabolism (Mattanovich et al. 2014, Thorwall et al. 2020, Geijer et al. 2022). By growing mutant pools under various stress conditions and with different carbon sources, tolerance to the stresses of industrial processing can be rapidly engineered and genotypes responsible for these traits can be uncovered. Finally, screens can be developed to manually select (Lupish et al. 2022), autonomously sort (Taguchi et al. 2023), or screen with biosensor reporting for high producers of valuable metabolites (D'oelsnitz et al. 2022, Robertson et al. 2024b).

Saccharomyces cerevisiae has been used for decades as a host microbe for metabolic engineering and as a representative of yeast biology due to its relative ease of transformation and high rate of homologous recombination (Nevoigt 2008, Parapouli et al. 2020). The Yeast Deletion Collection in particular proved valuable for functional genetic screens (Winzeler et al. 1999, Giaever et al. 2002, Giaever and Nislow 2014). CRISPR, an adaptive bacterial immune system, has been used as a tool for the past decade or so to rapidly modify all kingdoms of life. The widespread adoption

Received 29 November 2024; **revised** 12 March 2025; **accepted** 19 March 2025

of CRISPR-Cas systems for genome editing has made nonconventional yeasts, many of which are less genetically tractable than *S. cerevisiae*, more accessible, thus enabling new approaches to industrial biotechnology. One valuable approach is identifying a microbe that expresses a desired trait and adapt CRISPR genome editing to the selected species to control and enhance the desired trait(s) (Löbs et al. 2017, Patra et al. 2021, Geijer et al. 2022). Some examples of this approach include *Ogataea polymorpha* (Xie et al. 2024) and *Komagataella phaffii* (Claes et al. 2024), which are used for high protein production, *Kluyveromyces marxianus* is naturally thermotolerant and is used to produce valuable metabolites such as 2-phenylethanol and 2-phenylethyl acetate (Gao and Daugulis 2009, Li et al. 2021), *Rhodosporidium toruloides*, which produces lipids and carotenoids (Otoupal et al. 2019), and *Yarrowia lipolytica*, which accumulates high titers of lipids for applications in food or fuels (Blazeck et al. 2014, Schwartz et al. 2019). By developing functional genomic screens in these yeasts and others, we can expand their uses in industrial applications by screening for enhancements in valuable phenotypes.

In this review, we outline the current advances and historical development of functional genomic screens in yeast for biotechnology, emphasizing the use of CRISPR-Cas systems. We discuss these topics by selecting a subset of examples that best demonstrate the various technologies and approaches. We give a broad overview of methods and considerations for designing these screens as well as techniques to optimize their use. Since it is now possible to rapidly develop tools for nonconventional yeast, we highlight which nonconventional yeasts have been used in functional genomic screens and how these techniques were developed. Finally, we look to the future of yeast functional genomic screens, specifically how high-throughput automation and biosensors will be used to drive the selection of valuable phenotypes.

Approaches and methods to yeast functional genomic screening

Many genetic engineering methods can be used to generate mutations for functional genomic screening. Such methods are yeast deletion collections, RNAi, transposon insertional mutagenesis, and CRISPR-Cas (Table 1). In this section, we give an overview of these methods to build mutant libraries for the purpose of discovering valuable genotype–phenotype interactions.

The Yeast Deletion Collection

The Yeast Deletion Collection, the first yeast whole-genome knockout library (a single gene deletion per strain), was generated by Winzeler et al. (1999) in *S. cerevisiae* and was enabled by the complete sequencing of the *S. cerevisiae* genome (Goffeau et al. 1996) and the high homologous recombination efficiency of the host (Fig. 1A). This knockout collection is an arrayed set of mutant strains each with a single gene deletion and allows for the rapid testing of gene function. For example, the collection has been used to identify essential genes (i.e. those that greatly reduce cell fitness) in both rich and minimal media as well as genes that are essential for survival in particular stress conditions such as high osmotic stress, alternative carbon sources (Winzeler et al. 1999, Giaever et al. 2002, Giaever and Nislow 2014), or environmental stresses such as UV radiation (Birrell et al. 2001). Finally, these gene deletions can be multiplexed through double or triple knockouts to uncover genetic interactions (Giaever and Nislow 2014, Kuzmin et al. 2018, Liu et al. 2022b). The Yeast Deletion Collection is still used in current work and paved the way for functional genomic screens in yeast.

RNA interference

Since the discovery and functionalization of RNAi as a tool, its use has found diverse applications for synthetic biology in all kingdoms of life (Fire et al. 1998, Hannon 2002). In contrast to the yeast knockout collection, RNAi acts post-transcriptionally (on RNA, not DNA) to silence genes (Fig. 1B). Interestingly, the RNAi machinery is evolutionarily lost in some yeast, including *S. cerevisiae*, but can be reimplemented with the expression of the relevant protein machinery, often via plasmid expression (Agrawal et al. 2003, Drinnenberg et al. 2009). The use of a post-transcriptional gene interference technology presents additional challenges and opportunities when used in yeast (Hannon 2002, Chen et al. 2020). To our knowledge, RNAi technologies have not been demonstrated in *K. phaffii*, *Y. lipolytica*, *K. marxianus*, or *O. polymorpha*, only in *S. cerevisiae*. This is due in part to the more recent development of transformation protocols and basic genetic manipulation tools for many nonconventional yeasts.

Functional genomic screens using RNAi in *S. cerevisiae* have been widely successful. RNAi is advantageous in part because it allows for gene knockdown or activation as opposed to complete knockout. This allows for investigation of variable transcription levels and probing of essential genes (Si et al. 2015, Chen et al. 2020). Additionally, libraries can be generated from the genomic DNA of your host strain, rather than undergoing the costly process of gRNA or other DNA synthesis (Chen et al. 2020). Functional genomic screens using RNAi have successfully been used in *S. cerevisiae* to improve acetic acid tolerance (Si et al. 2015), isobutanol production (Si et al. 2017), and xylose utilization (HamediRad et al. 2018), among others. Some screens have been developed for both knockdown and activation simultaneously (Si et al. 2017) and some have been built for tunable knockdown (Crook et al. 2016).

RNAi applications on a genome-wide scale are not without obstacles. Constructing high-quality libraries demands rigorous quality control to ensure comprehensive genomic representation. Off-target effects remain a significant issue, often necessitating follow-up experiments to verify the reliability of identified targets. Furthermore, RNAi screens typically focus on phenotypes that are straightforward to measure, such as growth under chemical stress or substrate consumption, leaving more complex phenotypes underexplored. Introducing multiple RNAi reagents simultaneously can overwhelm the silencing machinery, reducing the efficiency of individual gene targeting. Additionally, understanding how combinations of mutations contribute to enhanced traits is challenging, requiring thorough studies to decipher their individual roles and interactions. Despite these challenges, the power of RNAi functional genomic screens suggests that this technology should be further explored for use in nonconventional yeast (Hannon 2002, Chen et al. 2020).

Transposon insertional mutagenesis

Transposons are portable genetic elements that insert themselves within chromosomes. As a synthetic biology tool, transposon insertional mutagenesis interrupts reading frames, promoters, noncoding regions, and other genetic elements nearly indiscriminately by inserting at TA or TTAA sites, depending on the system (Zhu et al. 2018) (Fig. 1C). After insertion, techniques such as inverse polymerase chain reaction (PCR) and randomly broken fragment PCR (RBF-PCR) can be used to amplify and then sequence the transposon and adjacent DNA to identify the insertion site (Näät-

Table 1. Comparison of functional genomic screening techniques.

Screen method	Advantages	Disadvantages
Deletion collections	Comprehensive coverage	Limited to nonessential genes
	Well-characterized	Complete knockout only
		Limited to *S. cerevisiae*
RNA interference	Can study essential genes	No complete knockout
	Variable gene repression	Transient effects
	Generated from host DNA	Off-target potential
Transposon insertion	Higher mutational depth	Insertion site bias
	Rapid development time	Random insertion limits scope
CRISPR-Cas	Precision editing	Off-target potential
	Versatile (KO, CRISPRi/a, base editor)	Library design and generation
	Can identify and study essential genes	Cellular burden from endonuclease

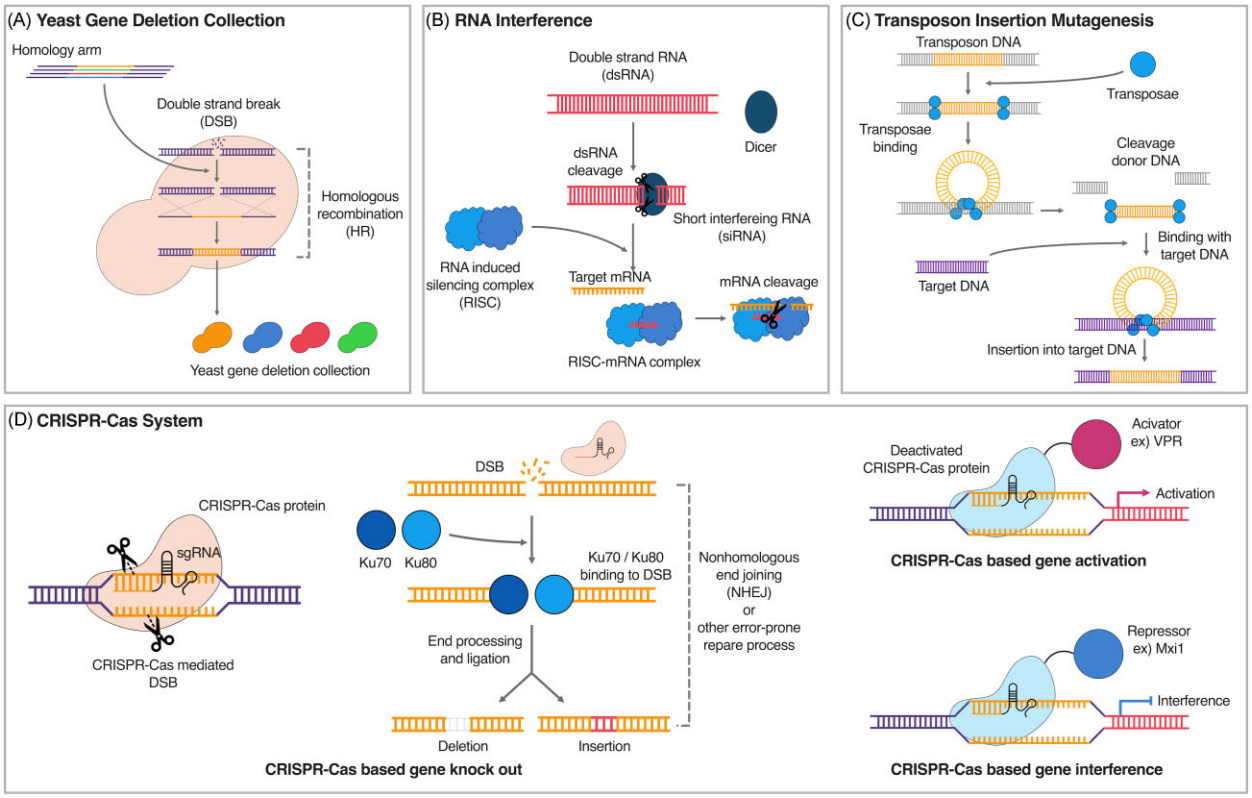

Figure 1. Common methods of generating mutations for functional genomic screens. (A) Yeast deletion collections utilize homologous recombination to generate gene knockouts. (B) RNAi occurs when Dicer protein cleaves double-stranded RNA (dsRNA) into small-interfering RNAs (siRNA), which are then incorporated into the RNA-induced silencing complex (RISC) to target and degrade complementary mRNA, thereby silencing gene expression. (C) Transposon mutant libraries rely on a transposase to randomly insert mutations throughout the genome. (D) The CRISPR-Cas system can be used to generate knockouts or up-/downregulate genes with CRISPRa/CRISPRi by using an sgRNA that directs a Cas endonuclease. In many cases, this double-stranded break (DSB) is then repaired by native error-prone repair enzymes like Ku70/80 creating an insertion or deletion that causes a premature stop codon downstream. For CRISPRa/CRISPRi, a dCas9 is fused to an activation/repression domain like VPR/Mxi1 to increase/decrease expression, respectively.

saari et al. 2012, Xu et al. 2013). Transposon insertional mutagenesis has been used for functional genomic screens in *S. cerevisiae* (Takahashi et al. 2001), *Schizosaccharomyces pombe* (Li et al. 2011), *K. phaffii* (Zhu et al. 2018), *R. toruloides* (Coradetti et al. 2018), and *Y. lipolytica* (Wagner et al. 2018), but work is missing in other nonconventional yeast.

Once a transposon insertional mutagenesis system is developed for a particular species, mutational libraries of the yeast can be created and screened. In one screen, *K. phaffii* colonies were individually picked and validated for their more efficient utilization of methanol as a carbon source (Zhu et al. 2018). In another, a transposon screen was developed for *S. pombe* and demonstrated by identifying genes related to microtubule formation and temperature sensitivity. Instead of picking individual mutants, up to 400 000 colonies were pooled and deep sequenced, demonstrating the throughput capabilities of the system (Li et al. 2011). Both of these projects utilized the *piggyBac* transposon system from the cabbage looper moth, *Trichoplusia ni*, due to its high transposition efficiency (Zhu et al. 2018). Finally, another study found two loci that improve growth on xylose with a transposon-based functional genomic screen in *S. cerevisiae* (Ni et al. 2007). The downside of transposon tools for functional genomics—the fact they are

untargeted—should be taken advantage of as it provides an opportunity to work with unsequenced genomes and rapidly implement the built tool because computational design is not needed before implementation. They can also be low or high throughput depending on the needs and the resources of the laboratory. Transposon insertional mutagenesis systems should be further developed, particularly for nonconventional yeast.

CRISPR-Cas systems

The advent of CRISPR-Cas tools has greatly accelerated the field of functional genomics due to the platform's flexibility (gene knockout, downregulation, upregulation, and base editing), portability between hosts, and programmability (Doudna and Charpentier 2014, Shalem et al. 2015, Anzalone et al. 2020, Trivedi et al. 2023). In a typical CRISPR-Cas system, an endonuclease is directed by gRNA (typically ∼20 bp in length) to make a targeted double-stranded break in the host genome. The cell repairs the break either through error-prone nonhomologous end joining (NHEJ) or microhomology-mediated end joining (MMEJ), which introduces insertions or deletions that disrupt gene function, or through homology-directed repair if a DNA template is provided enabling precise gene deletion or insertion (Schwartz et al. 2017, Xue and Greene 2021) (Fig. 1D). These repair mechanisms provide a foundation for creating targeted genetic alterations for functional genomic studies.

The versatility of the CRISPR-Cas systems extends beyond simple knockouts, as modifications of the Cas protein enable gene regulation without introducing double-stranded breaks. For instance, CRISPR interference (CRISPRi) utilizes a catalytically inactive Cas9 (dCas9) to block transcription by sterically hindering RNA polymerase at target genes (Qi et al. 2013). Conversely, CRISPR activation (CRISPRa) involves fusing dCas9 with transcriptional activators to enhance gene expression (Chavez et al. 2015) (Fig. 1D). These approaches provide a powerful toolkit for investigating gene function through precise modulation of gene activity. Moreover, some Cas proteins, like Cas12a (formerly Cpf1), exhibit distinct properties, such as the ability to process their own gRNAs and target multiple sites simultaneously using multiplexed sgRNAs (Ramesh et al. 2020). Limitations to CRISPR screens include ambiguous gene knockout scenarios (it is unknown whether frameshift-causing insertion or deletion is created), large size, cellular burden of the Cas9 endonuclease, discrepancies in gRNA activity predictions, and, like other screening methods, a need for high transformation efficiency. Despite these limitations and with advances in whole-genome sequencing and sgRNA library synthesis makes the CRISPR-Cas system a valuable candidate for functional genomic screens.

Optimizing CRISPR functional genomic screens

Despite the rapid development of the CRISPR-Cas system, further refinement is needed to optimize these technologies for genome-wide screening applications. Effective implementation demands extensive upfront work, including gRNA library design (Fig. 2). Library design criteria vary based on application (CRISPR knockout, CRISPRi, or CRISPRa). In knockout libraries, intronic regions are typically excluded, and guides are preferentially designed to target the first 5%–65% of the coding sequence to maximize the likelihood of a functional knockout. Alternatively, guides may target promoter regions to disrupt native transcriptional regulation, either through causing random sequence changes in the promoter regions with unmodified Cas endonuclease or by directing an activator or repressor to a specific site in CRISPRi/CRISPRa applications. Library design should also seek to address off-target effects. To this end, libraries should contain unique guides that are sufficiently spaced to improve diversity of target locations (Doench et al. 2016, Dong et al. 2021, Ramesh and Wheeldon 2021, Trivedi et al. 2023). sgRNA uniqueness within the genome minimizes off-target effects and enhances genome editing precision. Additional refinements can further optimize library efficiency. For instance, predicting sgRNA secondary structures can help avoid designs prone to forming stable secondary structures (Thyme et al. 2016, Labun et al. 2019), which may hinder complex formation with the Cas protein. Another key factor is the uniqueness of the sgRNA seed sequence (the 12–14 nucleotides upstream of the protospacer adjacent motif (PAM) site for Cas9), as mismatches outside this region are more tolerable, while seed region specificity is crucial for effective on-target activity (Jinek et al. 2012, Cong et al. 2013, Hsu et al. 2013, Jiang et al. 2013). While several sgRNA activity prediction tools exist, most are tailored for mammalian cells (Doench et al. 2014, Moreno-Mateos et al. 2015, Xu et al. 2015, Doench et al. 2016, Zhang et al. 2019), necessitating species-specific adaptations. One example of this is DeepGuide (Baisya et al. 2022), a machine learning-based guide prediction platform, has been introduced as an sgRNA design tool trained on experimental library data from *Y. lipolytica*. Finally, plasmid stability should be validated to ensure guide abundance changes are due solely to condition variations.

Given the challenges in predicting sgRNA activity, targeting multiple sgRNAs per gene increases the likelihood of successful gene disruption and improves fitness effect calculations. However, this also expands library size and complicates data analysis, particularly in hosts with limited transformation efficiency. One solution is to experimentally measure library activity by disrupting the dominant DNA repair pathway. In *Y. lipolytica*, *K. phaffii*, *K. marxianus*, and many other nonconventional yeasts (Löbs et al. 2017), the dominant repair mechanism is NHEJ. Knocking out key repair genes like *KU70/KU80* prevents DNA repair, causing cell death upon efficient CRISPR-induced cuts (Schwartz et al. 2019, Tafrishi et al. 2024). By comparing sgRNA abundance between an endonuclease-active strain and a control strain, sgRNA activity can be systematically assessed across the library (Robertson et al. 2024). An activity-validated sgRNA library consequently enhances the accuracy of genome-wide screens by ensuring that only functional guides contribute to phenotypic readouts. Investing in these foundational steps enhances the reliability of subsequent analyses, streamlines downstream processes, and accelerates the discovery of gene functions in the target host.

Applications of CRISPR genome-wide screens

Essential gene identification

Knockout of an essential gene causes cell death, stops cell growth, or substantially reduces growth rate. Gene essentiality may shift in different growth conditions, but there should exist substantial overlap between conditions and a core set of genes that are essential to growth in any condition. To identify essential genes with CRISPR genome-wide screens, populations containing the gRNA library and the Cas protein are grown and subcultured until the targeting and nontargeting control guides diverge in their abundance (Fig. 2). Many tools have been developed to predict gene essentiality from sgRNA abundance including acCRISPR (Ramesh et

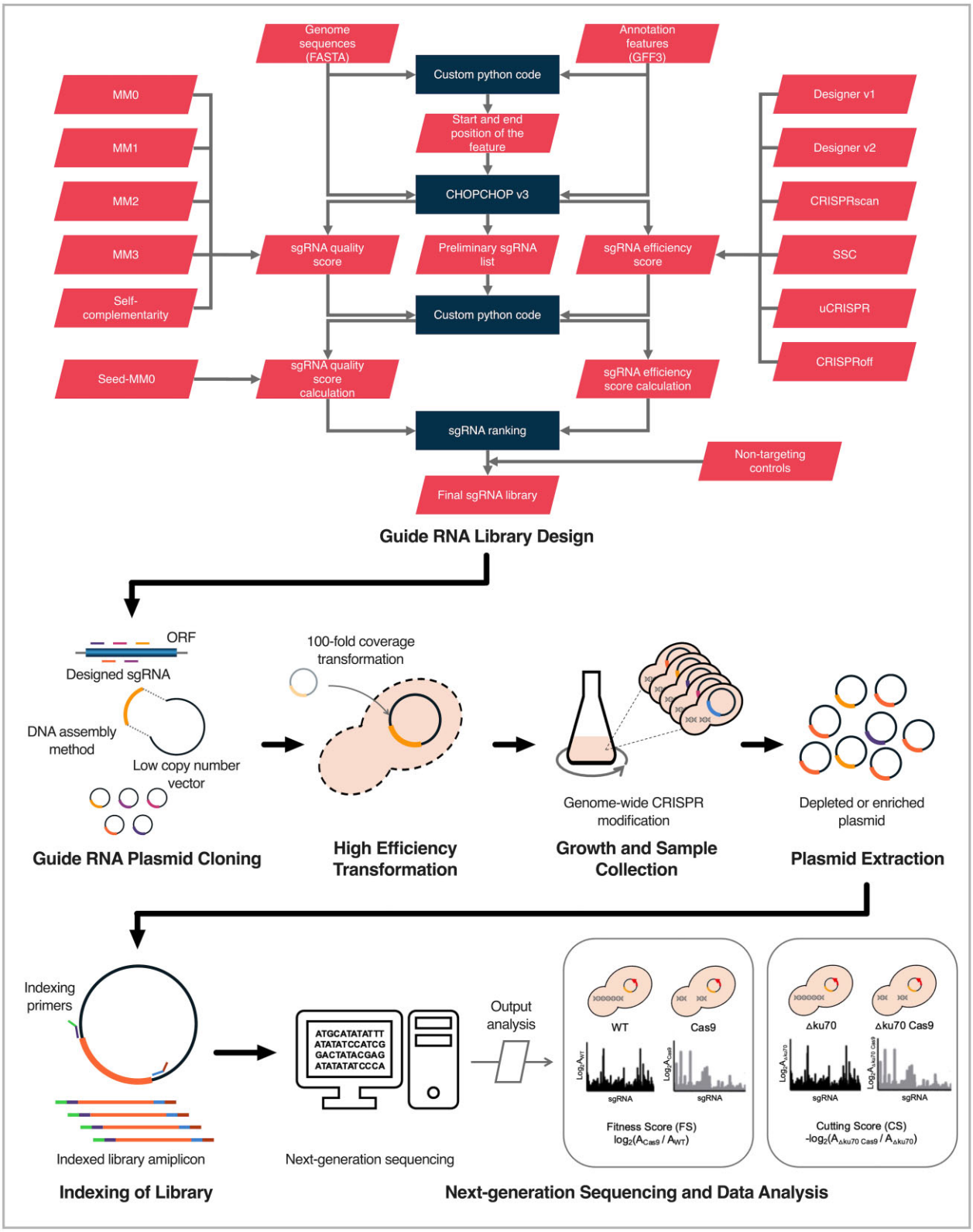

Figure 2. Pipeline for sgRNA library design and genome-wide screening with CRISPR-Cas systems. The CRISPR-Cas sgRNAs are predicted from annotated genomes of the target organism. Guide libraries are synthesized and cloned into, typically, low-copy number vectors to prevent multiple guide plasmids in a single cell. The transformed libraries are propagated in the given condition where strains with advantageous traits become enriched. After collecting plasmid DNA from each sample population, the sgRNA region is amplified with indexing primers required for later demultiplexing and next-generation sequencing. Finally, read counts are utilized to calculate guide or gene metrics like fitness score and cutting score.

Table 2. CRISPR-based functional genomic screens in yeast.

Screening	Species	CRISPR Tool	Target	Reference
Essential gene identification	*Y. lipolytica*	CRISPR knockout	Essential gene	Schwartz et al. (2019) and Robertson et al. (2024a)
	S. cerevisiae	CRISPR interference	Essential gene	Mcglincy et al. (2021)
	K. phaffii	CRISPR knockout	Essential gene	Zhu et al. (2018) and Tafrishi et al. (2024)
Stress tolerance-related gene identification	*S. cerevisiae*	CRISPR knockout	Furfural tolerance	Bao et al. (2018)
	S. cerevisiae	CRISPR interference	Absence of arginine, adenine	Momen-Roknabadi et al. (2020)
	S. cerevisiae	CRISPR interference	Acetic acid tolerance	Mukherjee et al. (2021)
	Y. lipolytica	CRISPR knockout	Canavanine tolerance	Schwartz et al. (2019)
	Y. lipolytica	CRISPR knockout	Salt tolerance	Ramesh et al. (2023)
	Y. lipolytica	CRISPR knockout	Acetate, fatty acid tolerance	Robertson et al. (2024a)
Biosensor-driven screens	*S. cerevisiae*	CRISPR interference	Acetic acid sensitivity	Mormino et al. (2022)
	K. marxianus	CRISPR knockout	Terpene	Robertson et al. (2024b)

al. 2023), developed and validated based on data obtained from yeast cells, and JACKS (Allen et al. 2019), MAGeCK-MLE (Li et al. 2015), and CRISPhieRmix (Daley et al. 2018) validated based on mammalian cell essential genes. Fitness scores of each gene can be calculated by averaging the change in abundance of each guide over time or comparing the abundance to a library transformed into a strain without the Cas protein; in our work, we define fitness score as $\log_2(A_{Cas9}/A_{wt})$, where A_{Cas9} is the given guide's abundance in the *Cas9* strain and A_{wt} is the given guide's abundance in the wild-type strain (Fig. 2) (Schwartz et al. 2019, Robertson et al. 2024a).

In *S. cerevisiae*, a CRISPRi library was able to call essential genes (Mcglincy et al. 2021). In *Y. lipolytica*, a first- and second-generation library were built and used to call essential genes (Schwartz et al. 2019, Robertson et al. 2024a). In *K. phaffii*, a CRISPR genome-wide screen revealed essential genes that overlap substantially with a previous transposon functional genomic screen. Through comparison of this essential gene set with that of other yeasts, a unique set of *K. phaffii*-exclusive essential genes were identified that were linked to this microorganism's nonconventional characteristics such as protein secretion and glycosylation (Zhu et al. 2018, Tafrishi et al. 2024). Essential gene information adds to our general understanding and paves the way to develop new tools. CRISPR function has been demonstrated in many other nonconventional yeast, paving the way for future CRISPR genome-wide functional genomic screens.

Stress tolerance-related gene identification

One variation on essential gene screens is stress-tolerance screens. In these cases, growth screens can be conducted in high salt or low pH media, on various carbon sources, or in other environmental stress conditions (Schwartz et al. 2019, Robertson et al. 2024a). Knockout of a low fitness score gene reduces fitness in the given condition whereas knockout of a high fitness score gene improves fitness in the given condition. Complexity increases when utilizing CRISPRi/CRISPRa to find tolerance-related genes or when altering promoter strength with CRISPR tools because guide activity (in the case of CRISPRi/CRISPRa) or promoter insertions and deletions (in the case of targeting promoter regions) vary transcription levels to unknown strength. With these methods, it is often the case that a gene can be flagged as important for a given condition, but the effect of changing the promoter strength is unknown, or a specific high-performing mutant needs to be isolated from the screen. To isolate a high-performing mutant from any style of screen, the stringency of the selection must be carefully designed. Highly stringent conditions may remove 99% of mutants, which is more amenable to selecting individual winners (positive screens), rather than deep sequencing. Less stringent conditions are more amenable to deep sequencing and to characterizing a spectrum of fitness effects. As with essential genes, these hits should be validated.

Stress tolerance screens have also been performed with nonconventional yeast, but there are considerably fewer examples than those conducted with *S. cerevisiae*. In *Y. lipolytica*, tolerance screens have been performed to identify genes related to canavanine resistance (Schwartz et al. 2019), salt tolerance (Ramesh et al. 2023), acetate tolerance, and fatty acid tolerance (Robertson et al. 2024a). These screens in *Y. lipolytica* demonstrate the enabling power of genome-wide screening to develop industrially relevant phenotypes.

Biosensor-driven screens

Screening for stress tolerance of a toxic product may improve yields, but tolerance is often not the limiting factor for metabolite production. Biosensor-driven screens bridge the gap between tolerance screens and production screens. A form of directed evolution, biosensor-driven CRISPR genome-wide screens, identifies genes responsible for improved metabolite production through the use of a biosensor. These screens could be set up with growth- or fluorescence-based reporting systems, for example.

Saccharomyces cerevisiae has been used to demonstrate a fluorescence-based acetic acid biosensor screen where a subset of genes were repressed with CRISPRi. Five genes were identified that, when repressed, led to higher acetic acid sensitivity (Mormino et al. 2022). In another work, *K. marxianus*, a PYR1 biosensor for the terpene geraniol, and a 10-fold coverage gRNA CRISPR-Cas9 knockout library were used to identify gene knockouts that improve geraniol production. Rather than fluorescence-based cell sorting, a growth-based system was used and individual colonies were picked for their size/faster growth rate, then were validated for improved terpene production (Robertson et al. 2024). In spite of the power of this system, few biosensor-driven genome-wide screening platforms have been developed. Additional compiled CRISPR functional genomic screens in yeast can be seen in Table 2.

Perspectives

With the rapid domestication of nonconventional yeast through the development of whole-genome sequencing, inclusive CRISPR tools, and improved transformation protocols, CRISPR functional genomic screens will become more prevalent in a broad range of yeasts. These nonconventional hosts already have attractive traits over baker's yeast that can be improved with these advanced screening systems (Geijer et al. 2022). Additionally, these yeast contain new or understudied genes that may be relevant for applications in industrial biotechnology. More advanced tools will allow for rapid multiround screens in strains that are already heavily modified for the process of interest, greatly accelerating strain development.

With more development in the field, guide activity will be more accurately predicted with larger experimental datasets and improved artificial intelligence/machine learning techniques that seek to generate predictive models of CRISPR activity and, more broadly, biological function. In many screening scenarios, sorting for hits is the bottleneck of elucidating valuable phenotypes (Mitchell et al. 2015, Lupish et al. 2022). The advancement of biosensors, like the PYR1 platform (Beltrán et al. 2022) and bacterial transcription factors (Tellechea-Luzardo et al. 2023), functional genomic screens will advance rapidly. With biosensor-driven screens, cells can self-report product titers allowing for easy sorting (D'oelsnitz et al. 2022, Robertson et al. 2024b). Current screening methods or these more advanced biosensor-driven screens can then be followed up with automation systems. Especially when paired with machine learning and machine vision, liquid handling robots can sort and test hits rapidly and full time (Torres-Acosta et al. 2022). The next decade of functional genomic screens will advance rapidly as other synthetic biology and computational tools develop.

Funding

This work was supported by the National Science Foundation (NSF-2323984, NSF-2225878 to I.W.).

Conflict of interest: None declared.

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Received 29 November 2024; **revised** 12 March 2025; **accepted** 19 March 2025