A prototrophic deletion mutant collection for yeast metabolomics and systems biology

To the Editor:
Auxotrophic markers—mutations in genes encoding enzymes in pathways for the biosynthesis of metabolic building blocks, such as amino acids or nucleotides—are used as selection markers in most yeast genetics and genomics experiments. The nutritional deficiency caused by the mutation (auxotrophy) can be compensated by supplying the required nutrient in the growth medium. This compensation, however, is not necessarily quantitative because such mutations influence several physiological parameters and may act in combination.2,4,5 The construction of a prototrophic derivative of the parent strain of the widely used genome-scale yeast deletion collection has confirmed the need to remove auxotrophic markers to reduce bias in physiological and metabolic studies. Moreover, flux balance analyses using a genome-wide metabolic model (Yeast 5) indicate that the activity status of some 200–300 reactions changes between different auxotrophic strains and the wild type. To alleviate this bias, we have constructed a version of the haploid deletion collection restoring prototrophy in the genetic background and thus preventing any influence of auxotrophy on the phenotype of a given gene deletion. This new deletion library is based on the popular S228c (MATα) knockout collection,6 and facilitates the exploitation of prototrophic yeast in both functional genomics and quantitative systems biology.

We assessed the physiological effect of auxotrophy by monitoring the growth of 16 yeast strains carrying all possible combinations of the markers (histidine (his3Δ); his3Δs06-495, his3Δ11, leucine (leu2Δ), methionine (met15Δ) and uracil (ura3Δ)) used in the MATα version of the yeast deletion collection.1 All markers and their combinations affected yeast growth, but without altering the adenylate (ATP, ADP and AMP) energy charge (Fig. 1a). As the most critical phenotypic quantity, the maximum specific growth rate (\( \mu_{\text{max}} \)) varied between 0.125 ± 0.003 (s.d.) h\(^{-1}\) (leu2Δ) and 0.207 ± 0.007 h\(^{-1}\) (ura3Δ his3Δ), rendering quantitative comparisons among these strains impossible (Fig. 1a and Supplementary Table 1). These growth differences could not be explained by the different medium supplementations for the following reasons: first, prototrophic yeast exhibited a different and substantially less diverse growth pattern in the 16 minimal media (Fig. 1b, left; medium recipes are given in the Supplementary Methods); and second, growth differences were altered, but not abrogated, when other proteogenic amino acids were included as well (synthetic complete medium; Fig. 1c).

On both types of medium, we observed complex (epistatic) interactions among all auxotrophic mutations. For instance, restoring MET15 had a negative effect on \( \mu_{\text{max}} \) in leu2Δura3Δhis3Δmet15Δ (0.185 ± 0.004 h\(^{-1}\) → 0.164 ± 0.003 h\(^{-1}\)) or leu2Δura3Δmet15Δ (0.162 ± 0.005 h\(^{-1}\) → 0.149 ± 0.001 h\(^{-1}\)) but, unexpectedly, promoted growth in leu2Δhis3Δmet15Δ (0.136 ± 0.006 h\(^{-1}\) → 0.173 ± 0.009 h\(^{-1}\); Fig. 1a); restoring LEU2 had a positive effect in leu2Δura3Δhis3Δ (0.164 ± 0.003 h\(^{-1}\) → 0.185 ± 0.006 h\(^{-1}\)) or leu2Δhis3Δmet15Δ (0.136 ± 0.006 h\(^{-1}\) → 0.161 ± 0.004 h\(^{-1}\)) but not in leu2Δura3Δhis3Δmet15Δ (0.185 ± 0.004 h\(^{-1}\) → 0.186 ± 0.007 h\(^{-1}\); Fig. 1a and Supplementary Table 1). Thus, although blocking different pathways, all markers affect one another, indicating that they have a wide-ranging and combinatorial influence on the metabolic network.

In batch culture experiments, further problems arise from the unequal consumption of amino acid supplements, resulting in cultivation phase–dependent
starvation. Growth of BY4741 (the auxotrophic parent of the standard yeast gene-deletion collection\(^1\)) in synthetic complete medium depleted nutrients such that they first became limiting for met15Δ, then for leu2Δ, his3Δ and finally ura3Δ auxotrophic yeasts (Fig. 1d). This effect could not be compensated for by increasing amino acid supplementation (s), as this inhibited cell growth (Fig. 1b, right).

Chronological lifespan (CLS) is a phenotype that is profoundly influenced by both nutrient supplementation and growth rate. Indeed, we observed an increase in stationary-phase survival in YPD medium upon restoration of prototrophy. In a competitive growth experiment, auxotrophic cells lost their colony-forming capacities within 10 days, but their prototrophic counterparts were fully viable for more than 20 days (Fig. 1e). Longer CLS of prototrophic versus auxotrophic yeast has also been reported for other genetic backgrounds, and nutrient starvation in synthetic media shortens the lifespans of auxotrophic cells\(^2,8\). Restoring prototrophy is thus one of the most powerful genetic modifications for extending CLS.

Thus, as auxotrophic markers have substantial and combinatorial epistatic influences on fundamental biological parameters, such as growth and aging, auxotrophic genome resources introduce bias for analyzing physiological parameters and even more so for quantitative studies addressing the metabolic network. We would thus encourage the yeast community to switch, where possible, to prototrophic yeast for experiments in transcriptomics, proteomics and metabolomics.

To create a prototrophic resource for genome-scale experiments, we reintroduced auxotrophic markers into the MATa versions of the S288c-based deletion collection (5,185 strains)\(^1\) and the titratable-promoter essential collection (839 strains)\(^3\). These strains were transformed with a centromere-containing single-copy vector (minichromosome) containing the chromosome VI centromere, the autonomous replication sequence of HHF1 (ARSH4)\(^9\) and the marker genes HIS3, URA3, LEU2 and MET15 under the control of their endogenous promoter sequences (pHLUM (Addgene ID 40276); Supplementary Fig. 1). Under nonselective conditions, the vector was transmitted in 99.15% of cell divisions (0.85% segregation mean over 20 generations). After 20 days, all cells were found to be prototrophic owing to their positive selection (Fig. 1e), facilitating screens on both selective and nonselective media. Furthermore, pHLUM- transformed BY4741 derivatives wild-type for HIS3, LEU2, MET15 or URA3 grew similarly to BY4741 pHLUM (Supplementary Fig. 2), indicating that

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**Figure 1** The combinatorial impact of yeast auxotrophic markers on yeast physiology. (a) Growth of 16 yeast strains carrying the auxotrophies of the MATa collection (his3Δ, leu2Δ, ura3Δ and met15Δ) in all possible combinations in the matching synthetic minimal media (n = 5; error bars, s.d.). Red bars indicate μ\(_{\text{max}}\) (Supplementary Table 1 for values), white bars the energy charge. OD, optical density; max., maximum. (b) Growth of prototrophic yeast in the 16 minimal media (left panel) or in minimal media with 10-fold increased supplementation of histidine (H), leucine (L), uracil (U) or methionine (M) (Supplementary Methods) (right panel) (n = 5; error bars, s.d.). (c) Growth of the 16 strains in synthetic complete medium (n = 5; error bars, s.d.). (d) Unequal amino acid consumption in batch cultures of BY4741. Media were recovered at the indicated BY4741 densities, resupplemented with YNB (nitrogen base) and glucose, and inoculated with the indicated auxotrophic strains. Background color indicates the final optical density reached after 35 hours. (e) Prototrophy increases stationary phase survival and selects for the pHLUM minichromosome. Cells were grown in YPD at 30 °C, plated at the indicated time points and replicated onto complete and selective media to determine auxotrophy. CFU, colony-forming units. (f) Auxotrophic markers cause synthetic lethality. Four examples are shown that were identified in a screen with the TET-off essential collection\(^2\). 13 (3.5%) of lethal phenotypes of doxycycline (DOX)-treated cells were rescued upon restoring prototrophy (Supplementary Table 2). ENO2 (a nonessential gene) and POL1 (essential in auxotrophic and prototrophic yeast) are shown as controls.
the minichromosome fully restored prototrophy.

We used the titratable-promoter essential collection\(^3\) to demonstrate screening capacities. By replicating original and prototrophic strains onto doxycycline-containing media, we found that 13 of the 370 lethal phenotypes were compensated (Fig. 1f and Supplementary Table 2). Thus, auxotrophic markers do not only influence physiological parameters, they are also responsible for several essential phenotypes.

Because all strains possess a native metabolic network, the new library reduces bias from the use of auxotropic markers in functional genomics and metabolic systems biology. On the basis of the pHLUM minichromosome, which is counterselectable, the new resource retains full compatibility with the popular S288c knockout and essential collections\(^1,3\). However, the use of a plasmid will introduce confounding factors to those mutants that have deficits in plasmid stability and segregation. The library is distributed as 96-well plate arrays (Euroscarf, Frankfurt) and contains a deep-red colored and counterselectable mutant (ade12Δ) on both universal and plate-specific positions, which simplifies plate orientation and identification and can serve as a replicate control in quantitative metabolomics experiments (Supplementary Fig. 3).

Note: Supplementary information is available at http://www.nature.com/doifinder/10.1038/nbt.2442.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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