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# A prototrophic deletion mutant collection for yeast metabolomics and systems biology

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Auxotrophic markers - mutations within genes encoding enzymes in pathways for the biosynthesis of metabolic building blocks, such as an amino acid or nucleotide, are used as selection markers in the vast majority of yeast genetics and genomics experiments [1-3]. 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 a number of 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[1] has confirmed the need to remove auxotrophic markers in order to reduce bias in physiological and metabolic studies [2] . Moreover, flux balance analyses using a genome-wide metabolic model (Yeast 5)[6] 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 deletant collection restoring prototrophy in the genetic background, such that influence of auxotrophy to the phenotype of a given gene deletion is prevented. This new deletant library facilitates the exploitation of yeast in both functional genomics and quantitative systems biology.

The physiological impact of auxotrophy was assessed by monitoring the growth of 16 yeast strains carrying all possible combinations of the markers (histidine (*his3Δ1*), leucine (*leu2Δ*), methionine (*met15Δ*) and uracil (*ura3Δ*) used in the *MAT***a** version of the yeast deletion collection[1]. All markers and their combinations affected yeast growth, but without altering the adenylate (ATP, ADP, AMP) energy charge (Fig 1 a). As the most critical phenotypic quantity, the maximum specific growth rate (μmax) varied between 0.125 $h^{-1}$ (*leu2Δ*) and 0.20 $h^{-1}$ (*his3Δmet15Δura3Δ*), rendering quantitative comparisons between these strains impossible (Fig 1 a, Suppl. Table 1). These growth differences were not explained by the different media supplementations, as *i)* prototrophic yeast exhibited a different and substantially less diverse growth pattern in the 16 minimal media (Fig 1c, left panel; media recipes are given in the Supplementary material); and *ii*) growth differences where altered, but not abrogated, when other proteogenic amino acids were supplemented as well (synthetic complete (SC) medium; Fig 1b). Importantly, on both types of media, complex interactions between all auxotrophic mutations were observed. For instance, restoring *MET15* in *leu2Δura3Δhis3Δmet15Δ* (0.185 $h^{-1}$ → 0.164 $h^{-1}$) or *leu2Δura3Δmet15Δ* (0.162 $h^{-1}$ → 0.149 $h^{-1}$) had a negative effect on μmax, but

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surprisingly promoted growth in *leu2Δhis3Δmet15Δ* (0.136 $h^{-1}$ → 0.173 $h^{-1}$) (Fig 1a); restoring *LEU2* in *leu2Δura3Δhis3Δ* (0.164 $h^{-1}$ → 0.185 $h^{-1}$) or *leu2Δura3Δmet15Δ* (0.136 $h^{-1}$ → 0.161 $h^{-1}$) had a positive effect, but not in *leu2Δura3Δhis3Δmet15Δ* (0.185 $h^{-1}$ → 0.186 $h^{-1}$) (Fig 1a; Suppl. Table 1). Thus, although blocking different pathways, all markers influence each other, 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 SC media depleted nutrients in a way they became first limiting for *met15Δ*, then for *leu2Δ*, *his3Δ1* and finally for *ura3Δ* auxotrophic yeast (Fig 1d). This effect could not be compensated by increasing amino acid supplementation(s), as this inhibited cell growth (Fig 1c, right panel).

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 media upon restoring prototrophy. In a competitive growth experiment, auxotrophic cells had lost their colony-forming capacities within 10 days, but their prototrophic counterparts were perfectly viable for more than 20 days (Fig 1e). Longer CLS of prototrophic versus auxotrophic yeast was also reported for other backgrounds, and in synthetic media nutrient starvation shortened the lifespan of auxotrophic cells [7,8]. Restoring protrotrophy is thus, to our knowledge, one of the most powerful genetic modifications for extending CLS.

Hence, as auxotrophic markers have substantial and combinatorial influences on fundamental biological parameters such as growth and ageing, auxotrophic genome resources introduce bias for analyzing physiological parameters and even more to 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 re-introduced auxotrophic markers into the *MAT***a** versions of the S288c-based deletion collection (5185 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 control of their endogenous promoter sequences (pHLUM; Suppl Fig 1, Addgene ID 40276). Under non-selective 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 prototrophic due to their positive selection (Fig 1e), facilitating screens on both selective and non-selective media. Furthermore, pHLUM- transformed BY4741 derivates wild type for *HIS3, LEU2, MET15 or URA3* grew similar as BY4741 pHLUM (Suppl. Fig 2), indicating that the minichromosome fully restored prototrophy.

The titratable-promoter essential collection[3] was exploited 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 1 f, Suppl. Table 2). Thus, auxotrophic markers do not only influence physiological parameters, they are also responsible for a number of essential phenotypes.

Since all strains possess a native metabolic network, the new library reduces bias from the use of auxotrophic markers in functional genomics and metabolic systems biology. Based on

the pHLUM minichromosome, which is counterselectable, the new resource retains full compatibility with the popular S288c knock-out and essential collections [1,3]. However, the use of a plasmid will introduce confounding factors to those mutants which have deficits in plasmid stability and segregation. The library is distributed as arrayed on 96-well plates (Euroscarf, Frankfurt), and contains a deep-red coloured and counter-selectable mutant (*ade12Δ*) on both universal and plate-specific positions, which simplifies plate orientation and identification, and can serve as replicate-control in quantitative metabolomics experiments (Suppl. Fig 3).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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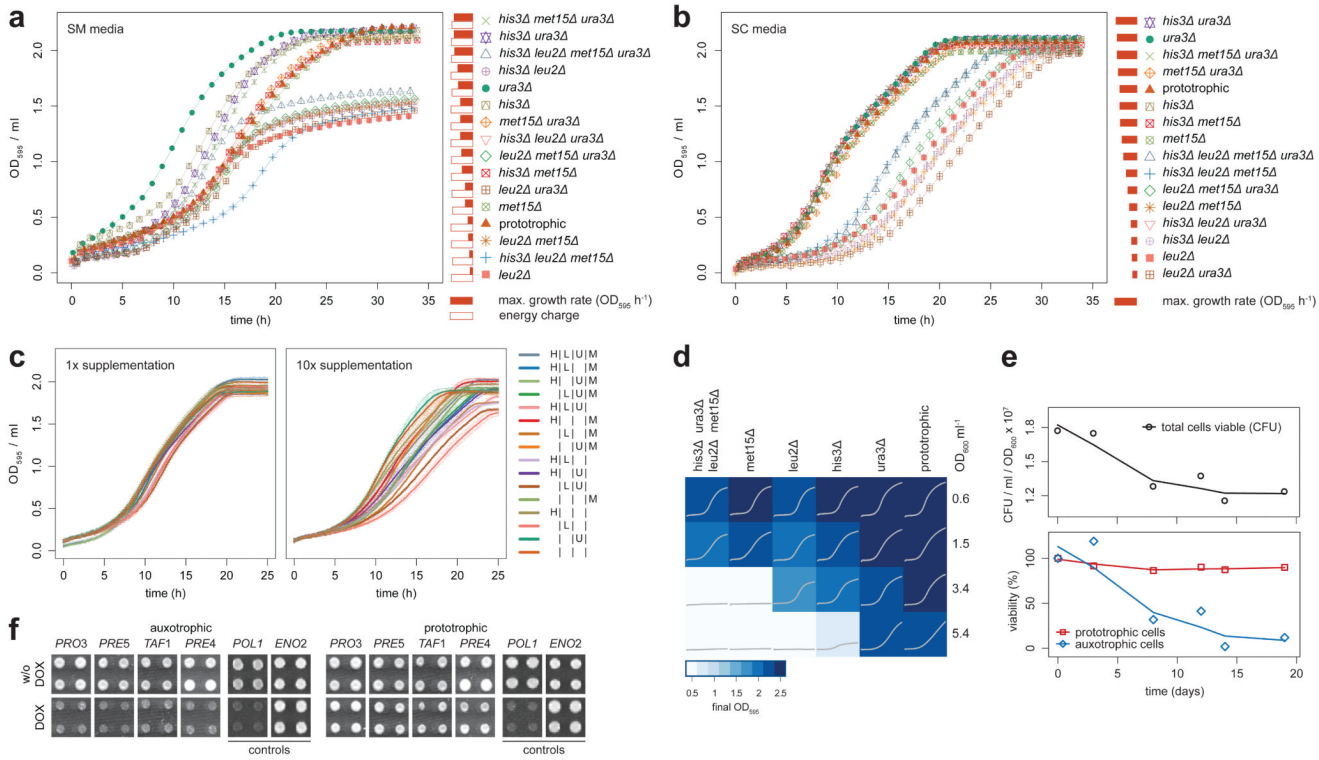


**Figure 1. The combinatorial impact of yeast auxotrophic markers**

***a)*** Growth of 16 yeast strains lacking the markers of the *MATa* collection (*his3Δ1, leu2Δ, ura3Δ and met15Δ)* in all possible combination in the matching synthetic minimal (SM) media ((n=5), Error bars +/− SD). Red bars indicate μmax (Suppl Table 1 for values), white bars the energy charge. b) Growth of the 16 strains in synthetic complete media ((n=5), Error bars +/− SD). ***c)*** Growth of prototrophic yeast in the 16 minimal media (left panel), or with 10x starting concentrations of histidine (H), leucine (L), uracil (U) or methionine (M) (right panel) ((n=5), Error bars +/− SD). ***d)*** Unequal amino-acid consumption in batch cultures of BY4741. Media were recovered at the indicated BY4741 densities, re-supplemented with YNB and glucose, and inoculated with the indicated auxotrophic strains. Blue background colours indicate the final OD reached ***e***) Prototrophy increases stationary phase survival, and positively selects for the pHLUM minichromosome. Cells were grown in YPD at 30C, plated at the indicated time points, and replicated onto complete and selective media to determine auxotrophy. ***f)*** Auxotrophic markers cause synthetic lethality. Four examples are shown that were identified in a screen with the *TET*-off essential collection[3], 13 (3.5%) of lethal phenotypes of DOX treated cells were rescued upon restoring prototrophy (Suppl Table 2). *ENO2* (non-essential gene), and *POL1* (essential in auxotrophic and prototrophic yeast) are shown as controls.