Construction of a Wine Yeast Genome Deletion Library

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Abstract:

Many of the important characteristics of wine, including alcohol content and flavour, are dependent on the strain of Saccharomyces cerevisiae used for the fermentation. These observed variations in the winemaking performance of various strains of S. cerevisiae are a direct reflection of genetic differences that are present in these strains. As laboratory strains, such as S288c, for which a genome deletion library is already available, perform poorly under winemaking conditions, it is impractical to use this resource to investigate the genes involved in wine fermentation. To address this shortcoming, we are constructing a genome-wide deletion library in a wine strain of S. cerevisiae. We have generated a haploid wine strain which retains the robust fermentation properties and wine metabolite and flavour profiles of its diploid parent strain. Each open reading frame in this haploid wine yeast is being systematically replaced with the corresponding deletion cassette obtained from the laboratory collection via PCR and homologous recombination. These cassettes each contain the KanMX selectable marker in addition to two unique molecular barcodes that allow for identification of individual genotypes from within complex mixtures of strains. When completed the library is expected to contain over 4000 deletion strains, with each mutant representing the loss of a single gene from the entire genome. Systematic screening of the library will allow for the identification of genes that make important contributions to the winemaking process and which can subsequently be used in the creation of novel strains with improved characteristics or with new combinations of winemaking characters. Currently the library contains over 2500 deletion strains and represents a valuable resource for investigating fermentation in the winemaking process.

Key words: wine, saccharomyces cerevisiae, genome