

# Optimization of the oleaginous phenotype in *Saccharomyces cerevisiae*

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Oleaginous yeasts are commonly defined by their ability to accumulate more than 20% lipid in their biomass. *Saccharomyces cerevisiae* belongs to the non-oleaginous yeasts, mainly due to the low lipid content of most laboratory strains, but also because it lacks the enzyme ATP:citrate lyase, which is thought to be required for oleaginicacy. However, neutral lipid accumulation is a quantitative trait and we found that the storage lipid content of different strain backgrounds of baker's yeast covers a broad range. By crossing two of the wild type strains with high lipid content and subsequent analysis of segregants, we obtained an oleaginous wild type of *S. cerevisiae*.

This strain was genetically engineered to further improve its triacylglycerol synthesis and storage properties. Several approaches were tested, including metabolic engineering of the lipid synthesis pathway itself, optimization of upstream reactions, modification of the redox balance, and the elimination of competing pathways. We combined the most efficient strategies in one strain and determined the minimal set of genetic modifications that is required for high lipid content.

This combination of genetic engineering, selection of the appropriate starting strain and cultivation under lipogenic conditions resulted in a mutant of *S. cerevisiae* with a triacylglycerol content similar to that of engineered oleaginous yeast species.