Project goals: to map genotype to phenotype on a genomic scale in the oleaginous yeast *Rhodosporidium toruloides*.

Wild isolates of the basidiomycete yeast *Rhodosporidium toruloides* can accumulate more than 70% of their biomass as neutral lipid in the form of triacylglycerol, and produce high concentrations of carotenoids. *R. toruloides* is also remarkable for its ability to utilize a wide spectrum of plant cell wall sugars and lignin derivatives and its natural tolerance of components of biomass hydrolysates that often inhibit growth of other species. These properties make *R. toruloides* an attractive host for sustainable production of hydrocarbon chemicals and fuels from lignocellulosic feedstocks. Although the *R. toruloides* research community is growing rapidly, relatively sparse biochemical data limits current engineering efforts. To enable the rapid interrogation of gene function on a genomic scale, we developed a 300,000+ member strain library of barcoded *R. toruloides* mutants via high efficiency *Agrobacterium tumefaciens* mediated transformation. With this mutant set we established methods for massively parallel assignment of phenotypes to genes in a given condition, by tracking the relative abundance of barcoded mutant strains in growth competition experiments and physical enrichments. We used this technique to identify a large cohort of genes (many with no homology to well-characterized genes in other systems) with roles in central carbon metabolism, nucleation and maturation of lipid bodies, protein trafficking, and nutrient sensing. Single-gene targeted deletion experiments validated the roles of these genes in the phenotypes inferred from our genomic approach. Together, these data provide a comprehensive understanding of the genetics of lipid metabolism in *R. toruloides*, and will serve as a foundation for the rational engineering of improved production strains of this fungus.

Funding statement: this project was funded by a grant from the Department of Energy Office of Biological and Environmental Research.