

Functional Genomics of Lipid Accumulation in *Rhodospiridium toruloides*

Dominic Pinel^{1#}, Samuel T. Coradetti^{2#}, Gina Geiselman¹, **Rachel B. Brem^{2,3}**, **Adam P. Arkin^{*1,4,5}** (aparkin@lbl.gov), and **Jeffrey M. Skerker^{1,4}**

¹Energy Biosciences Institute, UC Berkeley, Berkeley, CA; ²Buck Institute for Research on Aging, Novato, CA; ³Department of Plant and Microbial Biology, UC Berkeley, Berkeley, CA;

⁴Department of Bioengineering, UC Berkeley, Berkeley, CA; ⁵Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, CA

#These authors contributed equally to this project.

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

Wild isolates of the basidiomycete yeast *Rhodospiridium 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.

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