

## **Accelerating the engineering of improved lipid accumulation in *Yarrowia lipolytica***

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**Project Goal: Our goal is to enable rapid engineering of the oleaginous yeast, *Yarrowia lipolytica*, for increased rates of biofuel production.**

The oleaginous ascomycete yeast *Yarrowia lipolytica* serves as a model organism for lipid accumulation and the production of lipid based biofuels. In order to expand capabilities of *Y. lipolytica* for both biological research and industrial bioengineering applications, we have developed a set of genetic and molecular tools. In this work, strains were developed with increased homologous recombination, for targeted DNA incorporation. We have generated a library of fluorescent protein tagged strains to define organelle morphology in live cells (“The *Yarrowia* Organelle Atlas”) and built a set of plasmids to allow targeted overexpression and a *Yarrowia*-optimized GFP for fluorescent tagging. We have performed genome sequencing and assembly, and RNA expression analysis and transcript discovery in the PO1g background, which differs from the CLIB122 reference strain. This work provides the *Yarrowia* community with tools for the study of cell biology and metabolism of *Y. lipolytica* to further development for biofuels and natural products. In addition, we identified and tagged and overexpressed enzymes predicted to be important for the production of triglycerides from glucose with green fluorescent protein to identify their cellular location. Analysis of localization revealed that many enzymes are localized to the endoplasmic reticulum and lipid droplets. This localization pattern is not absolute. We observed two enzymes (Diacylglycerol acyltransferase, Dga1) and (1-acyl-sn-glycerol-3-phosphate acyltransferase, Slc1) that localize to both the endoplasmic reticulum and the periphery of lipid droplets.

### **Publications**

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2. Kerkhoven, E. Pomraning, K.R., Baker, S.E., Nielsen, J. 2016. Regulation of amino acid metabolism controls flux to lipid accumulation in *Yarrowia lipolytica*. *npj Systems Biology and Applications*, *In press*.
3. Pomraning, K., Baker, S.E. 2015. Draft genome sequence of the dimorphic yeast *Yarrowia lipolytica* strain W29. *Genome Announc.* 3(6). pii: e01211-15.
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