45. Developing a robust genetic and omic tool suite for the oleaginous yeast, *Yarrowia lipolytica*

Kyle R.Pomraning[1], Erin L. Bredeweg[1], Ziyu Dai[2], Mary S. Lipton[3], Eduard Kerkhoven[4], Jens Nielsen[4], Galya Orr[1], Thomas O. Metz,[3] and Scott E. Baker (scott.baker@pnnl.gov) [*1]

[1]Environmental Molecular Sciences Laboratory, [2]Energy and Environmental Directorate, [3]Fundamental and Computational Sciences Directorate, Pacific Northwest National Laboratory, Richland, Washington and [4]Chalmers University of Technology, Gothenburg, Sweden

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

Yarrowia lipolytica is a dimorphic oleaginous ascomycete yeast that produces high levels of lipids under nitrogen limiting conditions. We are developing a suite of genetic tools that allows for rapid and precise target gene deletion, overexpression and tagging. In order to maximize targeted transformation efficiency, we deleted a Y. lipolytica gene (ku70) involved in non-homologous end- joining DNA repair. To validate this system, we targeted four transcription factors for replacement with ura3. Split marker fragments for each were constructed and transformed into FKP424 (ura3, ku70::hph). Confirmation of gene replacement was confirmed by PCR amplifying across the gene of interest and looking for a size shift indicative of replacement with ura3. 5/6 transformants assayed had deletions which were further confirmed phenotypically. We have also collaborated with the PNNL Pan-omics program to identify and validate new Y. lipolytica genes for inclusion for metabolic modeling studies. Six enzyme codes were found not previously identified in Y. lipolytica annotations from KEGG or by annotation of the reference protein sequences with Blast2GO. Identification of candidate genes whose manipulation may lead to increased lipid accumulation in Y. lipolytica is accelerated by integration of- omic data with genetic engineering tools.

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