MOMENTUM: MICROBIAL OPTIMIZATION VIA METABOLIC NETWORK MINIMIZATION

Zhixia Ye, Duke University
zhixia.ye@duke.edu
Jonathan M. Burg, Duke University
Murphy R. Poplyk, Duke University
Eirik A. Moreb, Duke University
Ashley D. Trahan, Duke University
Daniel Rodriguez, Duke University
Wahab Sheikh, Duke University
Garrett Kelly, Duke University
Michelle Luo, North Carolina State University
Chase Beisel, North Carolina State University
Michael D. Lynch, Duke University

Key Words: Dynamic metabolic control, high-throughput metabolic engineering, metabolic network minimization, metabolic valves, robustness and scalability.

We report a high-throughput metabolic engineering platform enabling the rapid optimization of microbial production strains. The platform, which bridges a gap between current in vivo and in vitro bio-production approaches, relies on dynamic minimization of the active metabolic network and is implemented in the context of standardized 2-stage bio-processes. Dynamic metabolic network minimization is accomplished using combinations of CRISPR interference and controlled proteolysis to reduce the activity of multiple enzymes in essential central metabolism. This approach not only results in a design space with greatly reduced complexity, but also in increased metabolic fluxes and production rates as well as in strains which are robust to environmental conditions. Robustness leads to predictable scalability from high-throughput µL-scale screens, to fully instrumented L-scale bioreactors. Predictive high-throughput approaches are critical for metabolic engineering programs to truly take advantage of the rapidly increasing throughput and decreasing costs of synthetic biology. We have not only demonstrated proof of principle for this approach in two common industrial microbes: *E. coli* and *S. cerevisiae*, but also have validated this approach with the rapid optimization of *E. coli* strains producing two important industrial chemicals: alanine and mevalonic acid, at commercially meaningful rates, titers (147 g/L and 97 g/L, respectively), and yields.[1]

References:

Ye, Z., Burg, J.M., Poplyk, M.R., Moreb, E.A., Trahan, A.D., Rodrigiuez, D.L., Sheikh, W., Kelly, G.M., Luo, M.L., Beisel C.L., and Lynch, M.D. (2017) MOMENTuM: Microbial Optimization via MEtabolic NeTwork Minimization., Nature Biotechnology in review.