An Automated Sample Preparation Workflow For High-throughput, Quantitative Proteomic Studies of Microbes

Yan Chen,[1,2] Joel M. Guenther,[1,3] Jennifer W. Gin,[1,2] Leanne Jade G. Chan,[1,2] Zak Costello,[1,2] Tadeusz L. Ogorzalek,[1,2] Huu M. Tran,[1,3] Jacquelyn M. Blake-Hedges,[1,2,4] Jay D. Keasling,[1,2,5,6] Paul D. Adams,[1,2,5] Hector García Martín,[1,2] Nathan J. Hillson,[1,2] **Christopher J. Petzold** [1,2,*]

[1]Joint BioEnergy Institute, Emeryville, California
[2]Lawrence Berkeley National Laboratory, Berkeley, California
[3]Sandia National Laboratories (NTESS), Livermore, California
[4]Department of Chemistry, University of California Berkeley
[5]Department of Bioengineering, University of California Berkeley
[6]Department of Chemical and Biomolecular Engineering, University of California, Berkeley

www.jbei.org

Project goals: To develop an automated sample preparation workflow for quantitative proteomic analysis of engineered microbes.

Mass spectrometry-based quantitative proteomic analysis is key for biotechnology-related research and development. Driving this value have been improvements in sensitivity, resolution, and robustness of mass analyzers. However, manual sample preparation protocols are often a bottleneck for sample throughput and can lead to poor reproducibility. To alleviate these issues, we developed a "cells-to-peptides" automated workflow for Gram-negative bacteria and fungi that includes cell lysis, protein precipitation, resuspension, quantification, normalization, and tryptic digestion[1]. The workflow takes 2 hours to process 96 samples from cell pellets to the initiation of the tryptic digestion step and can process 384 samples in parallel. We measured the efficiency of protein extraction from various amounts of cell biomass and optimized the process for standard-flow LC-MS systems. The automated workflow was tested by preparing 96 Escherichia coli samples that resulted in a median coefficient of variation of 15.8% for over 600 peptides that were measured. Similar technical variance was observed for three other organisms as measured by highly multiplexed LC-MRM-MS acquisition methods. These results show that this automated sample preparation workflow provides robust, reproducible proteomic samples for high-throughput applications.

References

(1) Chen, Y.; Guenther, J. M.; Gin, J. W.; Chan, L. J. G.; Costello, Z.; Ogorzalek, T. L.; Tran, H. M.; Blake-Hedges, J. M.; Keasling, J. D.; Adams, P. D.; et al. Automated "Cells-To-Peptides" Sample Preparation Workflow for High-Throughput, Quantitative Proteomic Assays of Microbes. *J. Proteome Res.* **2019**, *18*, 3752–3761.

Funding statement: This work conducted by the Joint BioEnergy Institute was supported by the Office of Science, Office of Biological and Environmental Research, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.