Title: Development Of An Automated "Cells-To-Peptides" Sample Preparation Workflow For High-Throughput Quantitative Proteomic Applications

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Project Goals:

Recently, novel synthetic biology and metabolic engineering methods have enabled production of many valuable chemicals and advanced biofuel compounds. Mass spectrometry (MS)–based omics analysis has proven valuable for diagnosing the engineered organisms and aid metabolic pathway optimization. Innovative computational tools and recombinant DNA technology greatly speed up strain design and construction efforts, which places heavy demand on high throughput analytical platform. Several efforts have demonstrated that automated omics sample preparation could increase sample throughput and consistency. However, current effort mainly focused on automating procedures from proteins digestion to peptides cleaning up prior to LCMS analysis. Here, we developed a head-to-toe automated platform that prep are samples for omics analysis from biomaterial to LCMS analysis. Our goal of this complete automated platform is for high reproducible and less labor intensive omics sample preparation. This automation method could handle up to 384 samples in cell pellet form, and takes up to 5 hours prior to trypsin digestion. This method brings down the per sample cost to around $1.25. In addition, our automation method is the first-time report to prepare both proteomics and metabolomics samples from the same biomaterial. Our initial evaluation of the reproducibility of the automation platform were performed on quantitative proteomic analysis of a single 96 well plate samples. Our results showed that 88 % of 50 peptides from a total of 25 proteins have less than 20 % CV. The CV median was 10 %. We then extended our targets to 700 peptides out of 360 proteins, and our current result showed that the CV of 42.6% targets were below 20%, and the median CV of all targets was 21.8%. On the metabolomics side, we were able to consistently detect several common central carbon metabolites.

References:

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