Mechanistic Insights into Cell-free Gene Expression from an Integrated -Omics Analysis of Extract Preparation Methods

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Project Goals: We employ *E. coli* cell-free systems for enzyme synthesis and metabolic pathway prototyping to inform strain engineering in non-model organisms. Although cell-free gene expression has become a more common technique, relatively little is known about the shifts in metabolism and composition that occur when cells are lysed and processed to generate clarified cell extracts, which have different capacities for expression based on the specific steps employed during extract preparation. We sought to better characterize the behavior and composition of differentially processed extracts at the molecular level by performing kinetic analysis of transcription and translation in parallel with proteomic and metabolomic analysis.

Cell-free systems originated as simplified platforms for studying biological processes, and they have developed into widespread biotechnology tools for gene expression and high-throughput prototyping. Recent efforts have optimized cell-free systems from numerous organisms for applications such as biosensing, protein production, and metabolite synthesis by refining cell lysate preparation methods and cell-free reaction composition. However, the applied nature of this optimization toward improved rates or titers often limits investigation into the physiological mechanisms behind changes in process and chemical composition in cell-free synthetic biology. In this work, we assessed changes in transcription and translation activity for *E. coli* cell extracts prepared with acetate or glutamate buffer and the common post-lysis processing steps of a runoff incubation and dialysis. We applied proteomic and metabolomic analysis to uncover potential mechanisms behind these changes in gene expression with the processing steps separately and in tandem, highlighting the impact of runoff incubation on the proteome and the role of buffer composition on central metabolism. Better understanding the shifts in activity and composition of cell extracts will inform future cell-free biology efforts with significant implications for gene expression and biochemical conversion in pathway prototyping and biomanufacturing.

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