OPTIMIZATION OF E. COLI SOLUPRO™ USING SYNTHETIC BIOLOGY TO GENERATE A HIGH PERFORMANCE CHASSIS MICROBE FOR SCALABLE PRODUCTION OF PROTEIN THERAPEUTICS

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E. coli is a historically important research tool for early phase discovery and development of protein therapeutics. Nevertheless, Chinese Hamster Ovary (CHO) and other mammalian cell lines are the predominant production hosts for current generation antibody and antibody fragment production. Microbial hosts such as *E. coli* are used to produce a minority of approved biologic drugs relative to mammalian cell lines, due in part to perceived limitations in protein solubility and quality related to the complexities of protein folding, maturation, host specific post-translational modifications, as well as regulatory considerations. However, recent advances in our understanding of microbial biology and synthetic biology have enabled rapid progress to be made in the development of microbial cell lines that exceed the performance of best-in-class mammalian cell lines. AbSci has developed a functional reconstruction of the protein production environment of the eukaryotic endoplasmic reticulum in *E. coli*, which includes a semi-oxidized cytoplasm that facilitates appropriate protein folding and disulfide bond formation. Within the physiological context of *E. coli* SoluPro™, a best-in-class synthetic biology strategy that modulates rates of gene expression, protein expression, and protein folding using a plasmid-based design architecture have been validated as a strategy to produce soluble, high titer and quality protein biologics. Following construction of millions of plasmid variants using a pooled DNA construction library approach, plasmids are screened *in vivo* for improvements in protein titer and quality using a fluorescence activated cell sorting (FACS)-mediated antigen binding assay. Next generating sequencing (NGS) is used to identify genotypes enriched within populations of cells with enhanced antigen-binding properties. Secondary assays are performed to validate strain improvements identified by flow cytometry, including an orthogonal screening of antibody-mediated antigen-binding in cell lysates, as well as advanced Mass Spectrometry methods to quantify disulfide bond formation and other protein quality attributes. This strategy has enabled rapid identification of plasmid designs for soluble production of full-length antibodies and antibody fragments that can be scaled to multigram quantities of product in bioreactor fermentations of 48 hours or less. Additional optimization of the *E. coli* SoluPro™ chassis is being tailored to further improve folding and maturation of additional classes of complex therapeutic proteins. The ease of use of *E. coli* and technical robustness of our high-throughput discovery and optimization workflow enables AbSci to rapidly identify key conditions for heterologous protein production and identify protein folding solutions conditions that can exceed Gram level quantities of soluble protein with less than three months of strain optimization effort.