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Accurate scaleable microfermentation screening for microbial cell line development of therapeutic proteins

Erik Nordwald

Robert Todd

Tyler Telander

Aaron Pilling

Julius Johnson

ACCURATE, SCALABLE MICROFERMENTATION SCREENING FOR MICROBIAL CELL LINE DEVELOPMENT OF THERAPEUTIC PROTEINS

Erik Nordwald, KBI Biopharma, USA
enordwald@kbibiopharma.com
Robert Todd, KBI Biopharma, USA
Julius Johnson, KBI Biopharma, USA
Tyler Telander, KBI Biopharma, USA
Aaron Pilling, KBI Biopharma, USA

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The development of therapeutic proteins requires first selecting an appropriate cell line. In creating a cell line, both the microbial host strain and plasmid DNA sequence can have profound effects on the titer, purity profile, and overall manufacturability of a recombinant therapeutic protein. Choice of E. coli host strain can optimize titer, improve plasmid DNA stability, support disulfide formation, and/or minimize product-related impurities or degradation. Simultaneously, various plasmid elements including promoters, RBS, origins, tags, fusions, codon optimization strategies, and protein mutants can also affect titer, construct stability, purity, and overall manufacturability of the protein. Assessing these host strain-plasmid combinations should be done under representative fermentation conditions. Fermentation parameters may also need to be varied alongside the cell lines. We have implemented the Biolector microfermentation system to screen cell lines under relevant and varying fermentation conditions. This plate-based parallel microfermentation system can simultaneously screen cell lines while examining various fermentation parameters including media, feed-rates, pH profiles, and induction strategies. In one instance, we identified a cell line and fermentation strategy that improved upon the classical T7 (DE3)-system by more than ten-fold. After microfermenter screening, one or more strains may be further optimized in KBI's Ambr250 system. Although, integrating cell-line selection with fermentation development has streamlined subsequent development activities and even allowed direct scaling from the microfermenter to 10L stainless-steel or 30L single-use systems, in some cases. As a result of the integrated fermentation and cell line development, we are consistently exceeding 4 g/L titers after scale-up.

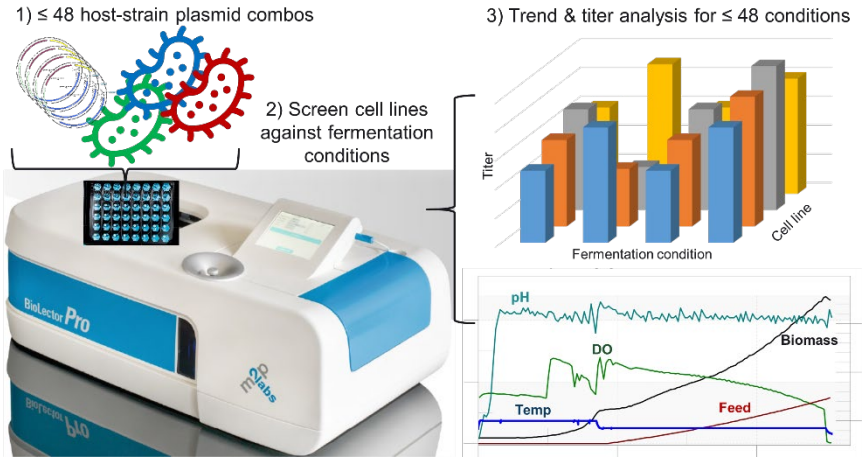

Figure 1. High-throughput microfermentation system integrates cell line development with fermentation development to optimize cell line selection. Full fermentation profiles for up to 48 wells are extracted from each experiment and that same number can be analyzed for titer and assessed for product quality by mass-spectroscopy.