IMPROVING TRANSIENT GENE EXPRESSION IN CHO-EBNA1 CELLS

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For pre-clinical evaluation of biotherapeutic candidates, protein production by transient gene expression (TGE) in Chinese Hamster Ovary (CHO) cells offers important advantages, including the capability of rapidly generating recombinant proteins that are highly similar to those produced in stable CHO clones used for biomanufacturing. The higher cost of reagents necessary for TGE, specifically the requirement for large amounts of purified DNA and transfection agent for each production, means that improving the performance of CHO TGE could substantially augment the method's utility. In the current study, we have established a novel CHO clone (CHO-3E7) expressing a form of the Epstein-Barr virus nuclear antigen-1 (EBNA-1). Transfection of EBNA-1-expressing cells with plasmid vectors encoding the Epstein-Barr virus OriP sequence boosted TGE productivity relative to parental CHO cells. Taking advantage of a new transfection-compatible media formulation that permits prolonged, high-density culture in shake flasks, we optimized transfection parameters (plasmid vector and polyethylenimine concentrations) and post-transfection culture conditions to establish a new, high-performing process for rapid protein production. The growth media is chemically defined, and a single hydrolysate feed is added at 24 h post-transfection, followed by periodic glucose supplementation. This method gave a maximum yield of 900 mg/L (for the chimeric IgG4 B72-3 mAb), with an average of 570 mg/L (standard deviation: 250 mg/L) for a panel of six mAbs and 320 mg/L (standard deviation: 140 mg/L) for five His-tagged recombinant proteins at 14 days post-transfection. Compared to our current low-density TGE process using CHO-3E7 cells and different culture medium, the new procedure gave on average 3-fold higher yields; purified mAbs produced using the two methods had distinct glycosylation profiles (by HILIC analysis) but showed identical target binding kinetics by SPR. Key advantages of the improved CHO-3E7-based protein production platform include the cost-effectiveness of the transfection reagent, the commercial availability of the culture media and the ability to perform high-cell-density transfection without media change.