DEVELOPMENT OF A HIGH THROUGHPUT CHO CELL GLYCOSYLATION ENZYME mRNA EXPRESSION PROFILER

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Glycosylation affects the physical and functional properties of recombinant glycoproteins and is a critical quality attribute that determines the efficacy of biologic drugs. Glycan profiles of biologics are a consequence of the interplay between the molecular sequence, the host cell line, and bioprocessing conditions. Differences in the relative activity of glycosyltransferase, sugar transporters, and other enzymes can cause variations in glycosylation profiles. The development of both novel biologics and biosimilars requires the generation of a cell line that expresses a protein with the desired glycosylation profiles. Consequently, high throughput glycan analytics and the use of this information for cell line creation and bioprocess optimization is essential. Thus, there is a need for a CHO-specific multiplexed glycan enzyme mRNA expression profiler. We have developed a simple, sensitive, and high throughput 96-well plate assay to probe 84 gene expression profiles in the CHO cell glycosylation pathway. This assay is based on branched DNA technology and uses signal amplification to measure mRNA transcripts. We deployed this approach to compare two CHO cell hosts expressing the same glycosylated protein. Our results indicated that the host expressing a highly mannosylated form of the protein had elevated mRNA expression of the mannosylation genes (Alg2, Alg3, Alg9, Alg11, and Alg12) and downregulation of glycosyltransferases Mgat1. This indicates that sub-optimal expression of these specific glycosylation genes results in glycosylation pathway bottlenecks that result in incomplete glycan processing. Another application of the high throughput assay developed in this study is the identification of differentially expressed glycosylation genes across bioprocessing conditions to guide process development and ultimately lead to a commercial manufacturing process that delivers the desired glycosylation profile. Overall, the high throughput assay developed in this study has broad applicability to study the glycosylation of CHO cells and can enable glycan-focused cell line and bioprocess development to optimize commercial manufacturing processes for biotherapeutic production.