SLOWLY CO-FLUCTUATING GENE EXPRESSION PATTERNS ARE HERITABLE AND ASSOCIATED WITH STRESS RESITANCE AND IMPROVED PRODUCTIVITY IN CHO CELL LINE DEVELOPMENT

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Many therapeutic proteins are produced using the Chinese hamster ovary (CHO) cell line due to their natural genetic plasticity, human-like post translational modifications, and superior production of secreted proteins. This genetic plasticity gives way to heterogenous clones that drive cell line development (CLD) where a monoclonal production cell line is identified based off optimized growth, productivity, and product quality. However, this CLD process represents a time and cost barrier to produce these therapeutics and is biased towards clonal populations that perform well in the unique environment that occurs during screening. It fails to identify optimal clones that perform exceptionally well in a larger production environment and associated stress agents. One approach for improving these CLD limitations involves narrowing the clonal pool based on biomarkers, which are genetic states that confer a favorable phenotype. This research describes a workflow for identifying heritable biomarkers slowly co-fluctuating and that are associated with stress resistance and improved productivity.

To identify suitable biomarkers, a population-based RNA sequencing technique, referred to as MemorySeq, was first used to identify gene expression states whose fluctuations continue for several divisions and were distinct from a bulk average noise control. These expression states are considered heritable if their variation significantly exceeded the transcriptome-wide variation in the bulk average noise control. Given the small number of cell divisions in this study, the gene expression fluctuations are likely epigenetically driven rather than the result of genetic mutations. These data were paired with differential gene expression analysis (DGEA) in the presence of stress characteristic of production conditions. The overlap of heritable expression states from MemorySeq and differentially expressed genes from DGEA with functional analysis may suggest genes that would bias the CLD clonal pool to better performance. The MemorySeq workflow identified nearly 200 heritable expression states and six network communities of co-fluctuating genes, characterized by cellular adhesion, response to chemicals and stimulus, and cell differentiation from GO enrichment analysis. High levels of ammonia, lactate, and osmolality were then introduced in fed-batch format to simulate production cycle media. Day 5 cell samples were used for DGEA and 130 of the heritable genes were differentially expressed in at least one of the stress conditions. Six genes associated with either higher protein secretion, negative regulation of apoptosis, or increased glycosylation were selected from this pool as possible biomarkers for screening. Clones with high expression of one or more of these six genes was selected and expanded for fed-batch culture to verify the heritability and assess its impact on production performance.