Improving productivity of CHO cell lines through genome editing, a better understanding of the epigenome and addition of hydrolysates

Abstract

Mammalian cells act as an ideal host for the production of recombinant proteins and therapeutics, with CHO cells being the most preferred. With increasing demands for protein therapeutics, it has become important to optimise CHO cell production. Optimisation can be achieved through genome engineering, understanding the epigenome of the cells or by supplementing the media with hydrolysates, thereby providing more nutrients for cell growth. The first study deals with the use of CRISPR-Cas9 tool to engineer CHO-K1 cells for an increased transient expression. A list of genes was identified by a previous study and gene knockouts were obtained using GFP-FACS/antibiotic resistance as selection pressure. The identified cell lines were found to have higher transient expression of IgG. The second study focusses on understanding the epigenome of cells pre- and post-gene insertion. Suspension adapted CHO-K1 cells secreting IgG were obtained and grown in culture. Genomic DNA was extracted from these cells and sequenced using Nanopore Sequencing, provided by Oxford Nanopore Technologies (ONT) MinION platform. Using the long reads obtained, the insert locations of the plasmid encoding IgG were identified by aligning the reads to the Chinese hamster reference genome. Multiple insertions, varying levels of average methylation were observed. When complete, ATAC-Seq performed on the CHO-IgG cells would provide a measure of gene expression. The third study involves hydrolysate supplementation with basal Excell CD CHO media to increase cellular growth and antibody titre of IgG expressing CHO cells. Cotton and soy hydrolysates were used and compared to basal media in batch cultures. An increase in specific growth rate and IgG titre was observed for the tested cotton hydrolysates. Proteomic analysis of the samples will provide further information on the proteins upregulated or downregulated in all three cases to account for the difference in growth and productivity.

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