CHARACTERISATION OF CHINESE HAMSTER OVARY (CHO) CELLS AT THE SINGLE CELL LEVEL

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Key Words: ImageStream, CHO, organelles, characterization

Biopharmaceuticals are a class of biological macromolecules that include antibodies and antibody derivatives, generally produced from cultured mammalian cells line via secretion directly into the media. Manufacturing requires the generation of Chinese hamster ovary (CHO) clonal cell lines capable of expressing the biopharmaceutical product at commercially relevant quantities with desirable product quality. The isolation of cell clones based on random single cell deposition via fluorescence activated cell sorting (FACS) provides a heterogeneous panel of expressers. We hypothesize that the application of FACS to provide an additional sorting step based on cell characteristics that correlate with productivity, product quality or cell growth attributes could lead to the isolation of higher producing cell lines with enhanced product quality attributes.

A panel of 20 cell lines expressing a model recombinant monoclonal antibody were characterised in terms of growth, productivity, and intracellular recombinant protein and mRNA amounts. Assays were also developed to investigate cell attributes and organelle content using the ImageStream instrument, an imaging flow cytometer, which enables the investigation of cellular characteristics that correlate with cell productivity at the single cell level.

Characterisation revealed the cell lines exhibited a range of values for productivity, growth, and intracellular (IC) antibody mRNA and protein expression, ideal for further ImageStream characterisation. Western blot and qRT-PCR analysis demonstrated that final titre correlated with both IC heavy chain (HC) protein and mRNA amounts (Pearson Correlation Coefficient (R) = 0.70 and R = 0.80, respectively). To assess productivity at the single cell level, assays multiplexing IC HC protein and mRNA with organelles, such as mitochondria, endoplasmic reticulum and Golgi apparatus, were therefore developed. ImageStream quantification of HC mRNA and protein amounts also showed correlations between titre and IC HC protein and mRNA (R = 0.84 and R = 0.79, respectively), confirming results from western blots and qRT-PCR analysis.

A cell attribute that correlates with specific productivity has been found, and current work is investigating whether this cell attribute could be used during cell sorting for the isolation of more productive clones. Future experiments will also look at cell attributes that could lead to improved product quality.

The developed assays are expected to allow a greater understanding of the intracellular mechanisms underlying productivity and product quality in CHO cells. Moreover, outcomes from this study have the potential to not only integrate into the cell line development clonal selection process, shortening timelines and reducing cost and resource requirements, but also inform host cell engineering projects with the potential for the development of an improved CHO host.