PROCESS FOR GENERATION OF HIGH-PRODUCING CHO CELL LINES FOR BIOMANUFACTURING OF BIOLOGICS USING OUR CHO^{rcTA} PLATFORM

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Chinese hamster ovary (CHO) cells are the most widely used mammalian host for industrial-scale production of mAbs and other protein biologics. Selection of high-producing cell lines is a major bottleneck in the process of manufacturing a novel biologic and requires an extensive and lengthy screening campaign of several hundreds of clonally-derived cell lines (clones). We have previously reported the development of a cell line (CHO^{rcTA}) containing an efficient inducible expression system, based on the cumate gene switch. Here, we present our method for selecting CHO cell lines using an automated approach. Stable CHO^{rcTA} pools are first generated following MSX selection. Fluorescently labeled cells are then deposited at one cell per well in 384-well plates using a cell sorter and pictures of each well are taken to assess monoclonality. Hundreds of cell lines are then screened in fed-batch in 96-deepwell plate format (0.5 mL) to identify top producers, which then enter expression stability study in a 20 mL format. High-producing, stable cell lines are then tested in 1-5 L bioreactors. Screening more than 25 different operating conditions in 96-deepwell plate format, we identified the condition which is most predictable of good performance in our 20 mL format. We demonstrate good correlation of productivity and predictability between the three different scales. We show that preventing protein expression during the pool selection step, as achieved with our inducible system, facilitates selection of high-producing cell lines. Imaging analysis provides >99% probability that selected cell lines were single-cell derived. We show data indicating that plasmid engineering allowed to increase cell line productivity by 75%, and that ~70% of selected cell lines have expression stability after at least 55 generations in culture. In two recent projects, productivity titers for Mab and Fc-fusion proteins using a generic process attained 3.4 and 2.8 g/L, respectively. Finally, we also present how we have engineered our platform for the production of afucosylated antibodies, and how we recently developed a selection approach allowing to select more productive CHO minipools prior to single cell cloning.