ENHANCING CHO CELL PRODUCTIVITY THROUGH A NOVEL DUAL SELECTION SYSTEM USING ASPG AND GS IN GLUTAMINE FREE MEDIUM

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The generation of high-producing Chinese hamster ovary (CHO) cell lines is achieved with selection methods such as dihydrofolate reductase (Dhfr) / methotrexate (MTX) or glutamine synthetase (Gs) / methionine sulfoximine (MSX). With increased understanding of CHO cell biology, it is possible to design new selection tools to add to the versatility of the toolbox. A recent pooled CRISPR-Cas9 screen revealed that Aspg is essential during glutamine deprivation. We hypothesized that it could work as a novel co-selectable marker in concert with the already widely used Gs selection system.

We generated CRISPR-Cas9 knockouts (KOs) of Gs, Aspg and a double KO of Gs/Aspg and transfected them with Gs-Enbrel and Aspg-Enbrel plasmids alone or simultaneously. Following selection in media without glutamine, we tested the cells at multiple points during expansion and compared their growth and productivity. Furthermore, we evaluated product quality and long-term stability.

Double KO cells transfected with both Gs-Enbrel and Aspg-Enbrel plasmids showed substantially improved specific productivity and improved titer compared to standard Gs selection method. While growth of these cell lines was initially low, adaptation improved viability and viable cell density while still maintaining a higher specific productivity and titer than Gs selection (Figure 1). Further optimization of media and selection conditions has the potential to further improve the growth phenotype, resulting in a considerable improvement over standard Gs CHO cell lines for industrial production of recombinant proteins.

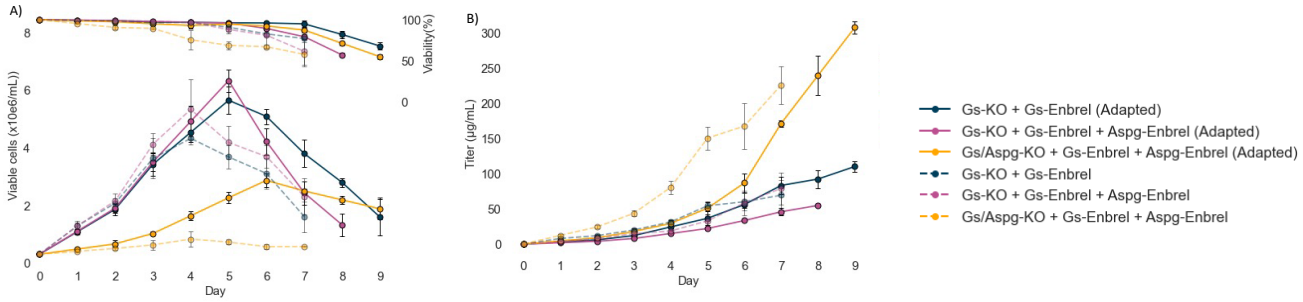

Figure 1 Shake flask batch culture of the top performing minipool from each transfection before and after adaptation showed improved viable cell density and titer. Viable cell density and viability (A) and titer (B). All cell lines were grown in three replicates. Error bars represent standard deviation.