Selection Process Development in a GS Knock-Out CHO Host Cell Line: The Effects of MSX Addition on Clones Generated in an MSX-Free Process

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Materials and Methods

Creation of IgG Producing Clones (MSX-Free, ACP Process)

Model IgG-producing stable pools were created using the CHOZ® GS-/- cell line (Figure 1), following the technical bulletin (SAFC). Two pools were single-cell cloned using limiting dilution, and 106 clones were evaluated. 7% of the clones achieved titers of more than 1×10^6 in a fed batch experiment. Nine high-producing clones were treated with MSX.

MSX Treatment

The nine IgG-producing clones were placed in media containing 25µM MSX and were cultured in TTP tubes. Following recovery (viability >97%), the MSX-treated clones were banked. During the MSX treatment, the clones were passaged to a VCD of 0.5×10^5 cells/ml twice weekly.

Fed Batch Assay (Volumetric Productivity)

Using the CHOZ® GS platform media and fed system, a fed batch assay was performed on the non-treated and MSX-treated clones, following the CHOZ® GS technical bulletin (SAFC). Titer was measured using the ForteBio Octet Platform.

Surface-Bound IgG

The MSX-treated and non-treated clones were stained using PE conjugated anti-human IgG washed and analyzed for surface-bound protein levels via MACSQuant Analyzer (Miltenyi Biotec). Gene Copy Number

The relative copy number of IgG Heavy Chain (HC) and GS genes of the clones was measured via quantitative PCR (Strategene Mx3000P). Relative gene copy numbers were determined using a standard curve created from serially diluted benchmark vector.

Results and Discussion

Effects of MSX Treatment on Clone Growth (Figure 2)

As shown in Figure 2, each clone responded differently to MSX addition in terms of growth. The minimum viability ranged from 46-92%, and the complete recovery time ranged from 17 to 27 days. No correlation was apparent between growth response to MSX addition and changes in peak titer, gene copy number or surface-bound IgG. For example, two clones that demonstrated the largest increase in volumetric productivity post-MSX treatment had the lowest titer (Clone 40) and the highest (Clone 34) minimum viability.

Productivity Changes Following MSX Treatment (Figure 4)

As shown in Figure 4, three clones showed significant increases in titer post-MSX amplification (Group 1). Three clones showed moderate increases (Group 2), and three clones showed reduced volumetric productivity (Group 3).

Relative Gene Copy Number Changes Post-MSX Addition (Figures 5-7)

In Group 1, only one clone showed a significant increase in HC copy number. This clone also showed an increase in GS copy number. In Group 2, no significant changes in HC or GS copy number were observed. In Group 3, two clones showed an increase in GS gene copy number, but a decrease in HC copy number. Overall, the relative gene copy numbers of GS and HC remained constant between the treated and non-treated clones.

Changes in Surface-Bound IgG (Figures 8-10)

In Group 1, all three clones showed a slight increase of surface-bound protein. In one clone, a second, non-producing population emerged post-amplification. In Group 2, one clone showed an increase in surface-bound IgG, but no significant change was observed in the other two clones. In Group 3 all clones showed a second population of non-producing cells that emerged post-amplification, which likely contributed to the decrease in volumetric productivity.

Stability of Non-Treated Clones (Figure 3)

The two clones with the largest decrease in productivity after 18 passages also showed decreased titer after MSX treatment. The surface-bound protein levels for these two clones also show emergent populations of non-producing cells after MSX addition.

Conclusions

- Glutamine (GS) selection is sufficient to isolate high-producing clones in CHOZ® GS-/-, but in some cases (3/9 clones in the present study), a 25µM MSX treatment can significantly increase volumetric productivity.
- In most clones, HC copy numbers did not trend with other indicators of gene amplification, such as surface-bound IgG or volumetric productivity, increased IgG productivity may be independent of HC copy number in these clones.
- Two clones demonstrated significant decreases in productivity but moderate increases in GS copy number. This implies that GS and IgG expression are decoupled in these clones, and MSX treatment is therefore ineffective.
- The two clones in which decoupling of GS and IgG expression was indicated also showed decreased titers after 18 passages in the absence of MSX.
- Our results indicate that increases in productivity observed post-MSX treatment are not a direct result of gene amplification. The exact mechanism is not completely understood, however it is apparent that this effect is clone dependent.

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References
