A PRODUCTION-ADAPTED, MULTI-AUXOTROPHIC, CHO CELL LINE FOR REDUCING MONOCLONAL AND BISPECIFIC ANTIBODY CELL LINE DEVELOPMENT TIMELINES

James Ravellette, Merck
james.ravellette@milliporesigma.com
David Razafsky, Merck
Mary Otto, Merck
Jason Gustin, Merck
Trissa Borgschulte, Merck

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Monoclonal antibodies (mAbs) have dominated the biotherapeutic landscape for decades and continue to be the predominant biotherapeutic modality on the market today. Bispecific antibodies (BsAbs), however, are the fastest growing biotherapeutic class, and will continue to be so, over the course of the next decade. Cell line development (CLD) for the production of traditional mAbs, requires significant investment of reagents, time, equipment, and personnel. These challenges are only magnified in the development of BsAbs, with hurdles such as low productivity, protein quality attributes, selection difficulties, and development timelines proving to be a significant hindrance to a drug candidate's speed to market (STM). To increase the STM and decrease the costs associated with the CLD process, we have developed a novel, production-adapted, multi-auxotrophic, CHO cell line capable of serving today's mAb-centric market, as well as the emerging BsAb market. Using a vector containing a lox-flanked (floxed) IgG expression cassette, driven by a glutamine synthetase (GS) selection marker, harboring a promoter trap, a high-producing clone was developed via random integration into the CHOZN® GS$^{-/-}$ cell line. Following characterization of the transgene integration event, as well as the growth, productivity, protein quality, and stability of this IgG expressing clone, we utilized the Cre-lox recombinase system to excise the floxed IgG expression cassette. This excision yielded a production-adapted host cell line (C1), harboring a single lox 'landing pad' (LP1) equipped with a promoter trap (Figure 1) that requires site-specific integration of transgenes at the target genomic locus. To enhance the utility of this cell line for BsAb production, introducing a second metabolic selection mechanism offered an elegant solution to overcoming the inefficiencies and drawbacks of current BsAb CLD selection methods, such as cotransfecting multiple vectors with identical selective markers, transfecting a single oversized plasmid (>20kb), or utilizing antibiotic-based selection. To this end, asparagine-depletion selection was identified as an ideal mechanism given that the synthesis of asparagine is a glutamine-dependent reaction. Zinc finger nucleases were designed to disrupt the endogenous asparagine synthetase (ASNS) gene in the C1 cell line, resulting in the CHOZN® GS$^{-/-}$ ASNS$^{-/-}$ clonal cell line (C2); a glutamine and asparagine auxotroph harboring LP1 (Figure 1). The utility of the C2 cell line, was validated via the cotransfection of vectors containing GFP and RFP driven by GS and ASNS selection markers respectively. Fluorescent protein expression in C2 cells, driven by either GS or ASNS selection, was significantly higher relative to the CHOZN® GS$^{-/-}$ host cell line, which is not production adapted. A second, promoter trap-equipped, lox 'landing pad' (LP2) was then introduced into a proprietary locus of the C2 cell line after which a clonal cell line was derived (C3). The resulting C3 cell line (Figure 1) is a production-adapted, multi-auxotrophic, CHO cell line containing two, promoter trap-equipped, landing pads capable of both high rates of site-specific integration and industry-relevant levels of recombinant protein expression. To demonstrate this cell line's utility, several vectors encoding mAbs and a BsAb were integrated into the landing pads of C3 and selected under multi-metabolic selection pressure. Transgene integration events, as well as the growth, productivity, protein quality, and stability of the mAb and BsAb expressing subclones was characterized. We demonstrate that the novel C3 cell line can produce stable, high recombinant protein-expressing cell lines in as little as 70 days, with a fraction of the hands-on time and resources currently required by the standard upstream cell line development process.

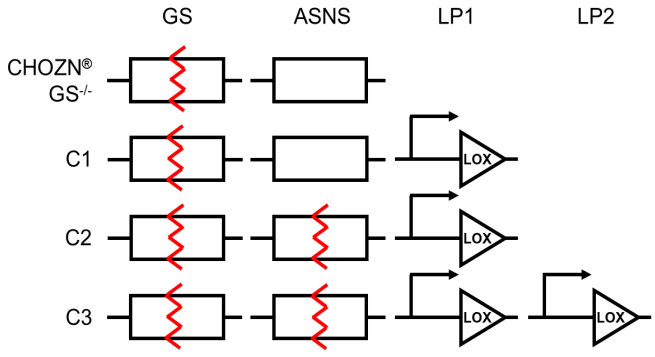

Figure 1. Cell line lineage and characteristics.
(Red markers indicate endogenous gene disruption)