WHITE PAPER

Leveraging next-generation cell line development technologies for cost-effective biotherapeutic applications

Introduction

Using biologically sourced material for biotherapeutics has achieved significant success in recent years, with transformative innovations throughout the entire cell line development (CLD) process. Automation, clone selection, scalability, and digitization have all contributed to increased agility, precision, and throughput while meeting regulatory compliance requirements.

Maintaining high-quality, safe, and effective processes is critical to the efficacy and safety of biotherapeutics. The complex nature of gene and stem cell therapies, monoclonal antibodies, bispecific and multispecific antibodies, nanobodies, recombinant proteins, and other customized novel biologic modalities means that the development and production of these medicines can be expensive. But large molecule treatment approaches have the potential to offer precise targeting capabilities and longer-term cost-effective results.

Biotherapeutic advancements for streamlined workflows

The importance of biotherapeutics continues to grow with increasing unmet medical needs in preventing and treating various disorders, including anemia, cancer, diabetes, as well as infectious and rheumatologic diseases. With the development and access to better technologies and processes, continued improvements to upstream and downstream stages of drug discovery and development workflows have been made to avoid factors that lead to poor quality, recalls, delays, or drug inefficacies. As a result, streamlined processes and best practices meet or exceed Critical Quality Attributes (CQAs) and hence, compliance requirements, during the development and production of of large drug molecules.

Chinese Hamster Ovary (CHO) cells are one of the most commonly used cell lines for the production of therapeutic proteins. They have benefitted from next-generation technological advancements in gene editing and enhanced expression levels.[1,2] Engineering CHO cells for various metabolic pathways has been shown to improve final product quality and enhance expression levels. Genetic editing tools such as CRISPR/Cas9 have facilitated significant breakthroughs in understanding gene functions and introducing specific modifications such as knock-out, knock-in, modulations, or integrating gene-of-interest (GOI).

Cell line development challenges

Clone instability, acceptable protein expression levels, and the overall time-consuming process involved in large molecule production are all continuing challenges in the race to market.

Random genetic host cell integration can introduce product variabilities at various stages of the CLD workflow, leading to instability, silencing of the transgene(s), or clonal heterogeneity even with the use of established clonal cell banks. Successful attempts at establishing well-characterized CHO cell line platforms earlier in the CLD process have led to better homogeneity in the final biotherapeutic product quality.[3-5]

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Addressing CLD issues with advanced technologies

Recent use of significant tools to overcome the random genetic integration are elements known as transposons. Transposon systems such as 'Sleeping Beauty' and 'PiggyBac' are used as agents to deliver integrating GOIs into active transcriptional sites on the host genome.[6] This enables faster CLD with higher titers of recombinant proteins, making transposons an ideal vehicle for the generation of stable expression cell lines.[7]

DNA transposons employ transposase activity and inverted terminal repeat (ITR) sequences to facilitate genomic integration. This "cut-and-paste" mechanism inserts transposon fragments into specific genomic sites to deliver recombinant genes.[8] To control the expression of the transposase gene, mRNA is often used. Transposon-based vector improves reproducibility between different transfections and clonal stability. In addition, due to its "cut-and-paste" mechanism, it nearly eliminates the risks of integrating bacterial elements as only the DNA within the ITRs is integrated into the host genome.[9]

CLD and large molecule expression workflows have adopted the use of well-established CHO cell lines and transposon-based technologies. These transposon expression systems allow for non-fragmented, multi-copy gene cassette integration.[10] This approach results in greater than 95% clonal stability at both the genetic and phenotypic levels, greatly reducing clone screening efforts and streamlining scale-up and process development.[11]

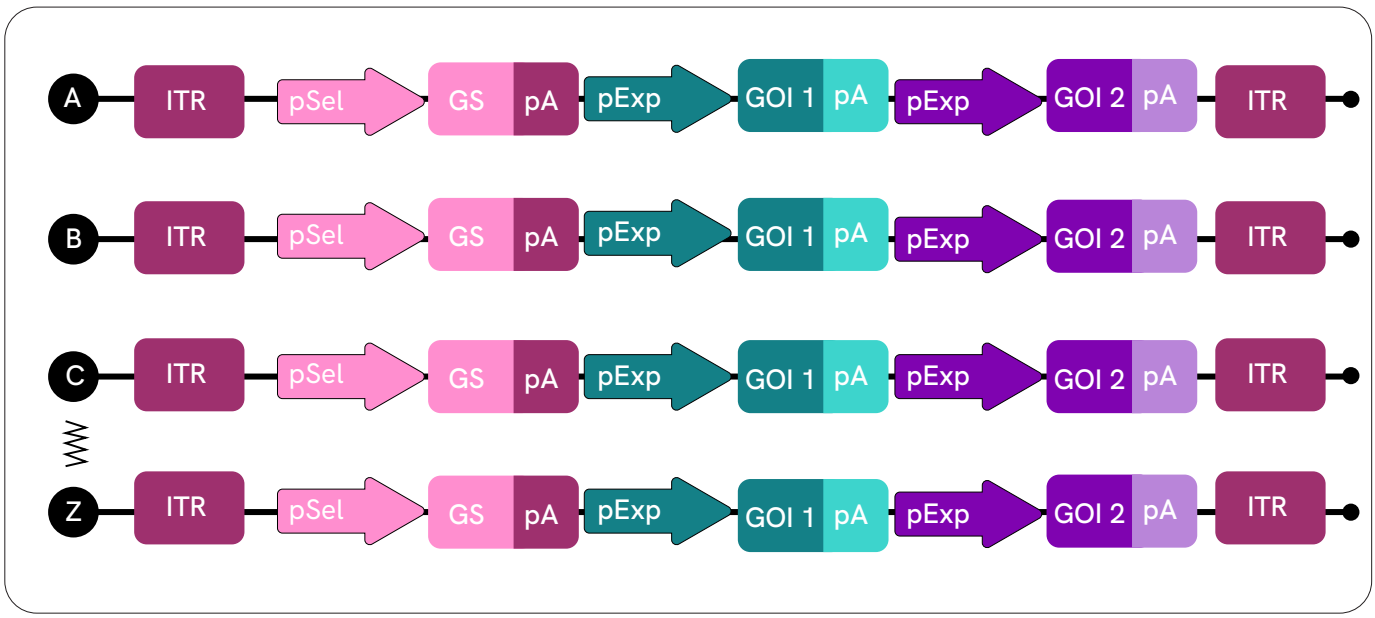

Figure 1: Transposon technologies offer simplified development and reduce manufacturing efforts. These transposon-based systems are devoid of any fragmentation and allow for the intact integration of the GOI at transcriptionally active sites (Figure 1), leading to homogeneity and increased clonal stability.

Conclusion

Large molecule CLD is a critical foundational step that significantly impacts product titer yields, scalability, elimination of cellular artifacts, and final biotherapeutic drug purification. Modern advances throughout the large molecule drug development workflow process have established more streamlined processes for Critical Quality Attributes (CQAs) to meet compliance requirements.

Transposon-based technologies offer more a cost-effective and simplified CLD process, reducing the time, costs, and effort needed for end-to-end manufacturing. Cell counting standardization and clone selection methods using automated cell evaluation technologies will further streamline both upstream and downstream stages of CLD.

Automated cell imaging, identification, detection, and analysis technologies are game-changers that greatly enhance accuracy and enable validation to facilitate best CLD practices, eventually contributing significantly to the entire biotherapeutics workflow resulting in greater yields and better efficacy and safety.

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Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com