WuXia™ TrueSite Targeted Integration Cell Line Development for High Protein Expression and an Accelerated Timeline

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Abstract

Typically, cell line development (CLD) relies on random integration and transposon-based integration, and both require intensive labor and can lead to exotic genes' instability. In recent years, the targeted integration (TI) expression system has emerged as an alternative CHO expression platform. TI allows for precise transgene insertion into the CHO host genome, resulting in recombinant cell lines with predictable transgene copies, genome location, and ultimately more predictable productivity with cell line stability.

Previously, the biggest hurdle to implementing a TI system for clinical and commercial manufacturing was the lower productivity. Recent advances in TI technology have demonstrated significant improvements in monoclonal antibody production yields [1,2]. At WuXi Biologics, we have developed a fully targeted integration system: WuXia TrueSite. The system represents state-of-the-art technology for next-generation CLD. In addition to delivering high product quality across a wide range of proteins, under platform fed-batch processes WuXia TrueSite routinely achieves clone productivity exceeding 8 g/L. Excellent cell line stability and consistency between pool- and clone-derived materials make it possible to produce non-GMP materials while eliminating dependence on cell line stability for final clone selection. This advantage streamlines CMC timelines to 6 months or less, enabling clients to accelerate best-in-class molecules into clinical development.

Methods

WuXia TrueSite host cells were derived from the WuXia™ CHO K1 cell line. Whole-genome sequencing of TI host cells identified the landing pad integration site, which was further verified by junction PCR and Sanger sequencing using integration site-specific primers.

TI host cells were co-electroporated with an expression vector harboring the gene of interest (GOI) and recombinase. The recovered pools and isolated clones were evaluated by platform fed-batch cultures in 50 mL spin tubes (TubeSpin Bioreactor 50). Cell cultures were harvested on day 14, and titers were determined using Protein A-HPLC. Cell harvests of spin tube cultures were 1-step Protein-A affinity purified using the Mabselect Sure LX column. Before analysis, purified samples were stored at 4 °C. The proteins were analyzed for SEC-UPLC, non-reducing Caliper purity (Caliper-NR), charge variants by capillary isoelectric focusing (cIEF), and N-glycan profiles by N-glycan-LC.

Results

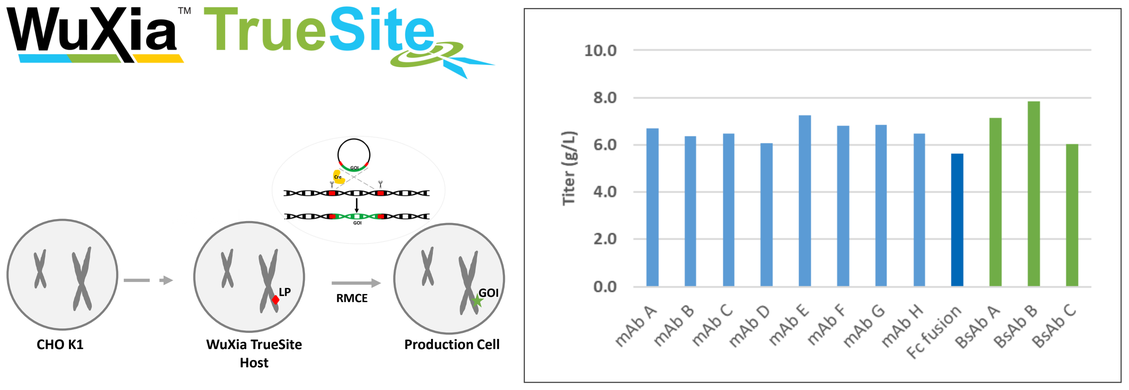

Figure 1. The schematic and performance of WuXia TrueSite

Current GMP-banked WuXia TrueSite host cells are derived from WuXia CHO-K1 with clear cell history. The GOI vector is integrated into the landing pad (GOI) through recombinase-mediated cassette exchange. WuXia TrueSite achieves pool titers over 6 g/L in a 14-day platform fed-batch process. After clooing, clone titers exceed 8 g/L. BsAb A is a 2-chain asymmetric antibody, while BsAb B and C are 3-chain and 4-chain asymmetric antibodies.

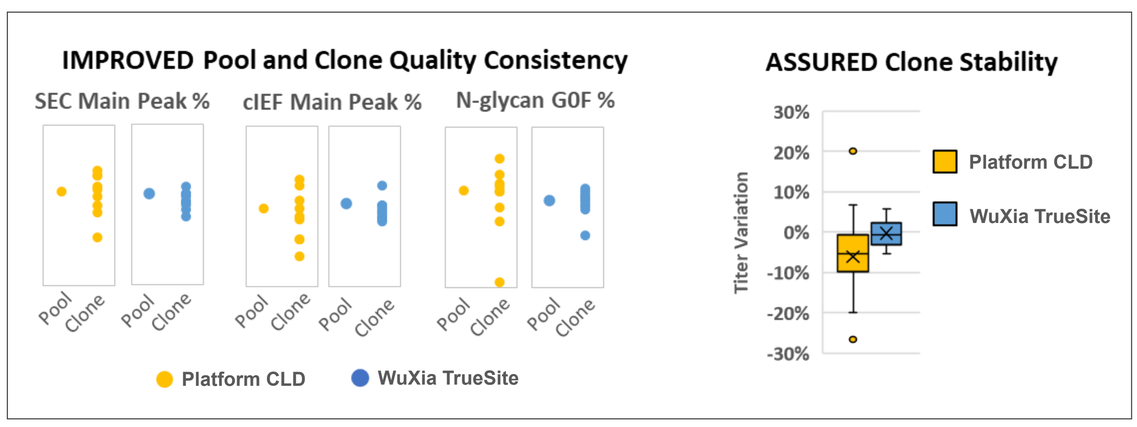

Figure 2: WuXia TrueSite for consistent quality and clonal cell line stability

Materials produced from WuXia TrueSite clonal cell lines exhibit greater uniformity with pool products compared to platform CLD processes. All tested WuXia TrueSite clones demonstrate stable performance within ±10% beyond 60 population doubling levels after cell banking.

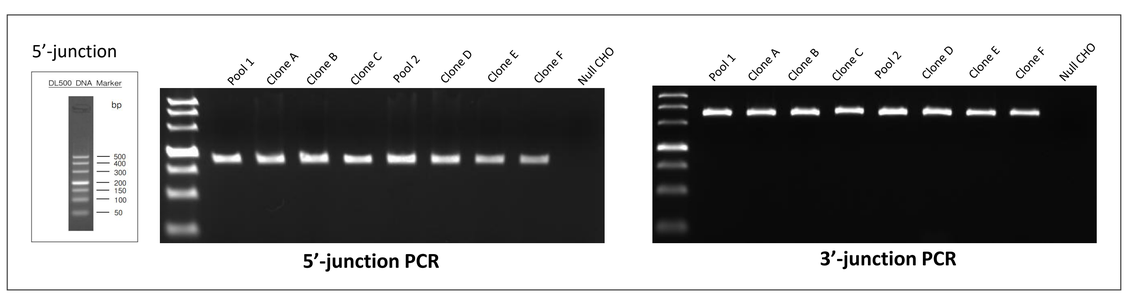

Figure 3: WuXia TrueSite integration site uniformity

5′ junction PCR and 3′ junction PCR using primers located on genome and plasmid. Clones A, B, and C were derived from Pool 1, and clones D, E, and F from Pool 2.

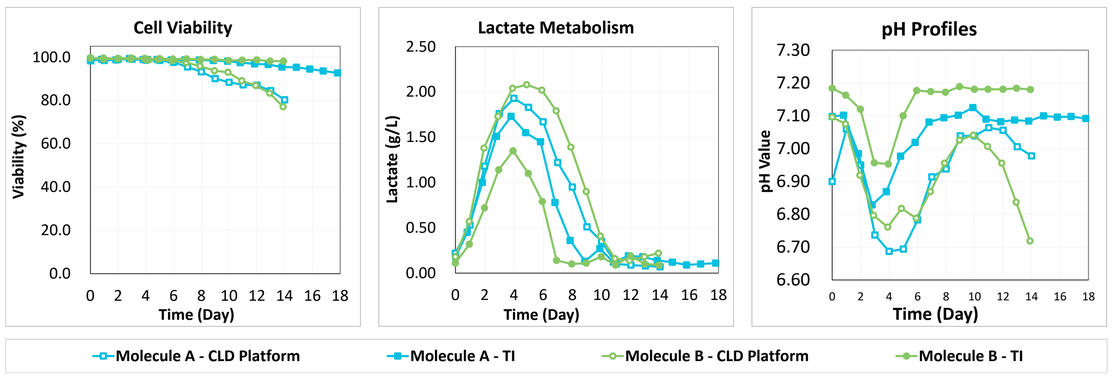

Figure 4. WuXia TrueSite improved VIA, Lac, and pH profiles for easy scale-up

Clones selected using WuXia TrueSite maintain higher viability during bioreactor cultures, even when the duration is extended to 18 days. Additionally, they exhibit improved lactate consumption and a more stable pH. These results indicate that WuXia TrueSite is better suited for large-scale culture.

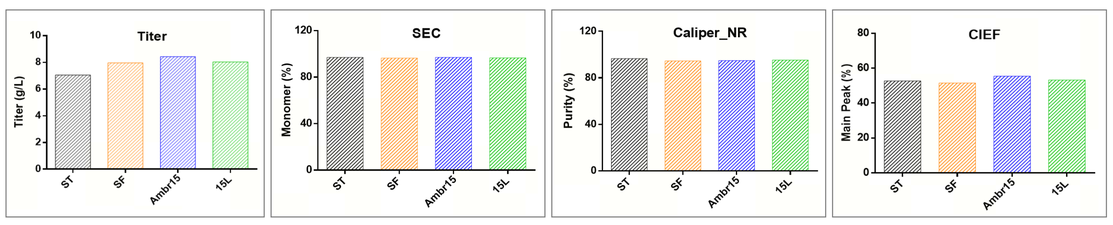

Figure 5. Highly consistency productivity and PQAs across culture scales and models

Overall, clones selected through the WuXia TrueSite strategy demonstrated highly consistent productivity and product quality attributes (PQAs) across a spinning tube, a shake flask, Ambr15, and 15 L bioreactor cultures, indicating great scalability.

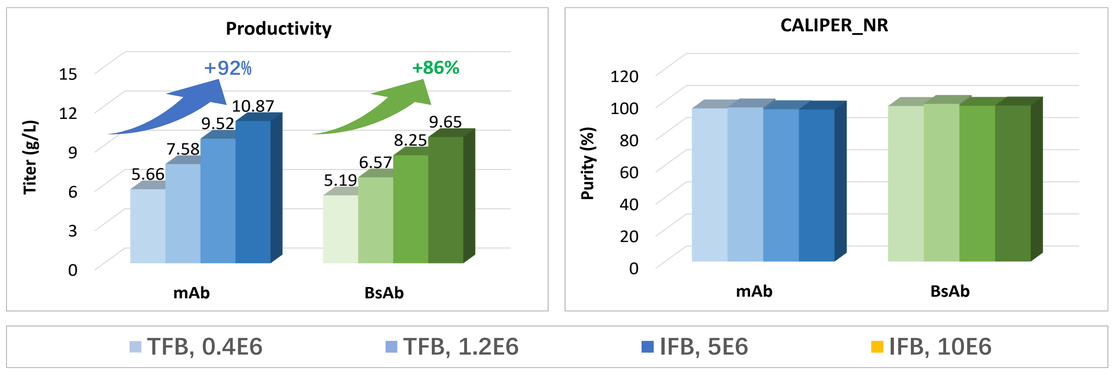

Figure 6. High potential for improving productivity via intensification

Clones selected via WuXia TrueSite exhibited improvements in VIA and viable cell density, along with favorable lactate metabolism and pH. Consequently, productivity can be enhanced using intensified strategy. TFB: fed-batch; IFB: intensified fed-batch; E6: seeding density of 1×10^6 cells/mL.

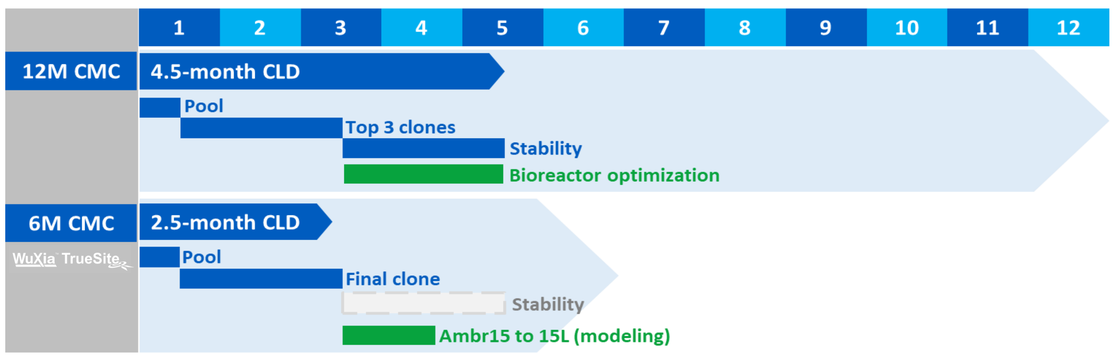

Figure 7: Reshaping the CMC timeline with WuXia TrueSite

WuXia TrueSite shortens the CMC timeline to as little as 6 months by using pools for non-GMP material generation and eliminating dependence on cell line stability for final clone selection. As a result, WuXia TrueSite enables clients to accelerate advancement of best-in-class molecules into clinical development.

Conclusion

WuXi Biologics developed the WuXia TrueSite system, which enables high productivity and accelerates CMC development. Under initial (non-optimized) fed-batch conditions, WuXia TrueSite supported antibody productivity over 6.0 g/L of stable pools and 8.0 g/L of clones in 15 L bioreactors. With a clear characterization of host cells, seamless process scalability from Ambr15 to 15 L scale with high modelling accuracy was achieved through upstream efforts. As very few contract research, development, and manufacturing organizations (CRDMOs) provide TI CLD service, we aim to enable our clients with best-in-class molecules along a ≤ 6-month IND timeline, or demanding an upgraded cell line with improved productivity and stability.

References

1. Ng, D.; Zhou, M.; Zhan, D.; Yip, S.; Ko, P.; Yim, M.; Modrusan, Z.; Joly, J.; Snedecor, B.; Laird, M. W.; Shen, A. Development of a Targeted Integration Chinese Hamster Ovary Host Directly Targeting Either One or Two Vectors Simultaneously to a Single Locus Using the Cre/Lox Recombinase-Mediated Cassette Exchange System. Biotechnology Progress 2021, 37 (4), e3140.

2. Beal, K. M.; Bandara, K. R.; Ali, S. R.; Sonak, R. G.; Barnes, M. R.; Scarcelli, J. J.; Zhang, L. The Impact of Expression Vector Position on Transgene Transcription Allows for Rational Expression Vector Design in a Targeted Integration System. Biotechnology Journal 2023, 18 (9), 2300038.

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WuXi Biologics is a global leading CRDMO offering end-to-end solutions to enable partners to discover, develop, and manufacture biologics from concept to commercialization.

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