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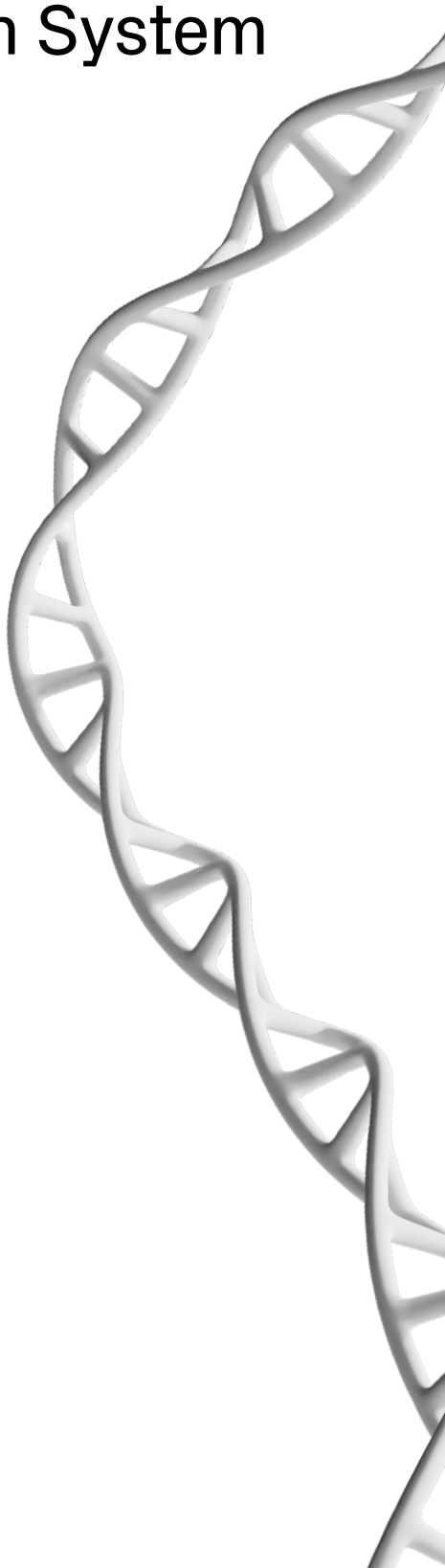
Application Note
Bioprocessing With TcBuster

Enhancing Cell Line Development With the TcBuster™ Transposon System

Utilizing TcBuster to Increase Antibody Titer in CHO-K1 Clones vs. Random Integration



Development of stable, highly productive cell lines is a critical step in the production of therapeutic antibodies. Engineering these cell lines is commonly performed by random integration, resulting in a heterogeneous pool from which many cells must be single cell cloned, expanded, and screened to identify high titer clones that are stable over time in culture. This process can take several months to a year and is labor intensive, requiring hundreds of clones to be screened to develop optimal cell lines (1, 2).

TcBuster is a DNA transposon-based system that allows for stable gene transfer into virtually any cell type. The system, composed of TcBuster-M mRNA transposase and DNA transposon containing the gene of interest (GOI), is electroporated into the cell. TcBuster transposase is then translated, binds to inverted terminal repeats (ITRs) on either side of the genes of interest on the transposon, and excises and inserts the GOI randomly into the host genome (3).

This study compares the production of CD28 antibody-producing CHO-K1 cell lines generated either through random integration or TcBuster-mediated transposition. Cell lines are developed through selection, single cell cloning and analysis to identify clones with the highest antibody titer and copy number. The top-producing clones are cultured over several weeks, and the analysis is repeated to assess the stability of the clones over time. Finally, one clone from each pool is cultured in an Ambr® 250 high throughput bioreactor to determine antibody production under various culture conditions.

Key Takeaways

- Use of the TcBuster system resulted in a stable pool that expanded quickly under selection, with 5-fold higher antibody titers and increased average copy number compared to the pool generated through random integration
- Top clones from the TcBuster pool had antibody titers > 500 mg/L compared to < 100 mg/L for the random integration clones, offering potential time and cost savings by requiring fewer clones to be screened to achieve desired results
- Top clones derived from the TcBuster pool consistently produced more CD28 antibody than top clones from the random integration pool over 30 doublings (46 days) in culture
- One clone from each pool cultured in a high throughput bioreactor for 14 days resulted in a maximum of 12 g/L of antibody produced from the TcBuster clone, compared to a maximum of 0.6 g/L without TcBuster

Materials

MATERIAL	SUPPLIER	CATALOG NUMBER
TcBuster-M 001.1 mRNA	Bio-Techne	TCB-001.1-100
TcBuster-M Compatible DNA Plasmid (encoding for CD28 antibody and puromycin resistance)	In-House	N/A
HyClone™ ActiPro™ Cell Culture Media	Cytiva	SH31039.03
HyClone CDM4MAb Cell Culture Media	Cytiva	SH30802
HyClone Cell Boost™ 7a Supplement	Cytiva	SH31119.01
HyClone Cell Boost 7b Supplement	Cytiva	SH31120.02
InstiGRO™ CHO Plus	Advanced Instruments®	NC1668724
Anti-Clumping Agent	Gibco	0010057 AE
CD CHO Medium	Gibco	10743029
Dynamis™ Medium	Gibco	A2661501
EfficientFeed™ B+ 3X Supplement	Gibco	A3937501
Glucose Solution	Gibco	A2494001
GlutaMAX™	Gibco	35050079
Puromycin Dihydrochloride	Sigma-Aldrich®	P9620
Neon™ Electroporation System	Thermo Fisher	MPS100
Neon Transfection System 100 µL Kit	Thermo Fisher	MPK10025
IMMUNO-TEK™ Human IgG ELISA	ZeptoMetrix	0801182

Table 1. Materials and Reagents used in this study.

Experimental Workflow

The workflow for generating CHO-K1 clones is depicted in Figure 1. Initial cell pools were produced by electroporating CHO-K1 cells using the Neon transfection system. Two pools were generated: 1) the random integration pool, containing CHO-K1 cells electroporated with the CD28 antibody-encoding transposon, and 2) the TcBuster pool, containing CHO-K1 cells electroporated with the transposon and TcBuster mRNA. After two days of recovery, pools underwent puromycin selection, which continued for 33 days to ensure that cells were fully selected. After selection, stable pools were analyzed for inserted copy number using digital PCR and antibody titer via IgG ELISA. Pools were then single cell cloned by limiting dilution. Once clones were established, clones were screened for copy number and antibody titer and the top three clones from each pool were selected based on highest titer. Clones were cultured for 46 days (30 doublings), and analysis was repeated to assess stability of the clones over time in culture. Additionally, one clone from each pool was cultured in the Ambr 250 high throughput bioreactor for 14 days under varying culture conditions. The time from electroporation to initial clone screen was 11 weeks, and the total culture time from electroporation to conclusion of the Ambr study was 19 weeks. Therefore, the TcBuster workflow offers significant time savings compared to classic cell line development, which typically takes an average of 24-28 weeks from vector synthesis to final release (4).

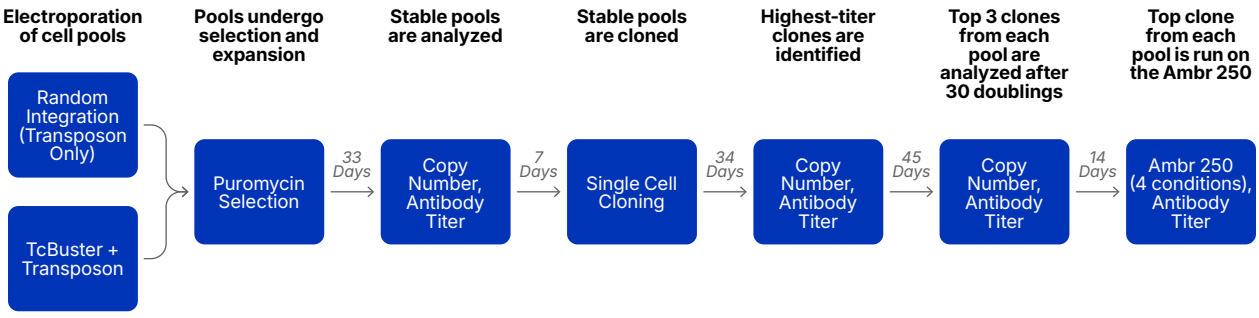


Figure 1. Workflow for engineering, selection, cloning and analysis of CHO-K1 cell lines.

Culture and Bioreactor Conditions

Two media formulations were used throughout generation of clones and stability studies. Growth medium, composed of CD CHO medium supplemented with 6 mM GlutaMAX and 0.5% Anti-Clumping Agent, was used for development of stable pools, cloning, and initial clone screening, between the 0 and 30 doubling timepoints of the stability study. Production medium, consisting of HyClone CDM4MAb supplemented with 6 mM GlutaMAX and 0.5% Anti-Clumping Agent, was used for the 7-10 day antibody titer studies. Both media formulations are chemically defined and protein- and serum-free. Throughout this study, CHO-K1 cells were cultured at 37 °C and 5% CO_2 on an orbital shaker kept between 90-120 rpm.

The Ambr 250 bioreactor experiment included two media formulations: Dynamis medium supplemented with Gibco Efficient Feed B+ and glucose or ActiPro medium supplemented with CellBoost 7a and 7b. All fed batch reactor vessels were inoculated at 0.5 x 10^6 cells/mL with temperature set to 36.5 °C. Supplements were administered by the Ambr throughout the 14-day run according to manufacturer specifications for each culture medium. Antifoam was added as needed, and pH was set to 7.0 and controlled throughout the production run. Four bioreactor conditions were tested in duplicate for each clone: each media formulation kept at 36.5 °C throughout the entire run, and each media formulation with the temperature shifted to 33 °C on day 5.

Results

Generation of Stable Pools using TcBuster vs. Random Integration

Figure 2 shows the development and analysis of CHO-K1 pools. CHO-K1 pools were genetically modified utilizing either TcBuster or random integration. Stable pools were generated by selecting pools with 6 µg/mL puromycin for 33 days prior to analysis. The random integration pool was sensitive to selection due to low gene integration, so a longer selection time with lower puromycin was utilized. Cumulative fold expansion of CHO-K1 pools was tracked throughout selection. Over time, the TcBuster pool had higher fold expansion than the random integration pool and lost fewer cells during puromycin selection **(2A)**. Following selection, stable pools were grown in 6 well plates for 7 days before analysis of CD28 antibody titer via IgG ELISA, which showed that the cell pool generated using TcBuster-M transposase had a 5-fold increase in antibody titer compared to random integration **(2B)**. An increase in integrated copy number, determined by digital PCR, was seen for the cell pool generated using TcBuster transposase compared to random integration **(2C)**. Overall, the TcBuster stable pool showed increased fold expansion, antibody titer, and copy number compared to the random integration pool.

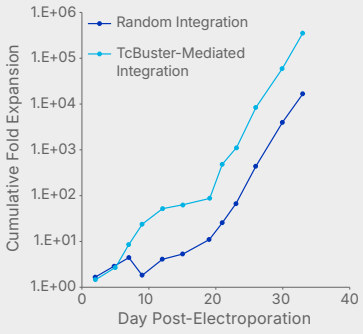


Figure 2A. Fold Expansion

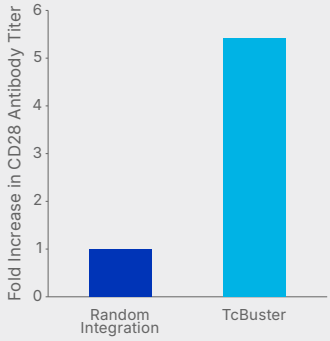


Figure 2B. Fold Increase in Titer

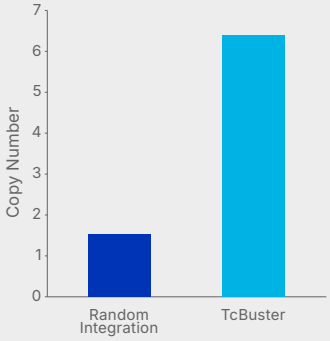


Figure 2C. Copy Number

Figure 2. TcBuster-M transposase-mediated gene editing yields stable pools with higher antibody titers and inserted copies than plasmid alone.

Top-performing Clones Resulting from TcBuster and Random Integration Pools

Stable CHO-K1 pools were single cell cloned in 96-well plates by limiting dilution. When cultured in serum-free media, CHO-K1 cells are sensitive to single cell cloning which may limit the number of clones that can be achieved per plate. To aid in cloning, single cells were supplemented with a CHO cloning additive to support clones until they were expanded beyond the 96 well plate. Cloning efforts resulted in 58 clones from the random integration pool and 72 clones from the TcBuster pool across two 96 well plates each. 15 random integration clones and 17 TcBuster clones were screened for CD28 antibody titer and copy number and the top 3 clones with highest antibody titer from each pool were expanded and further characterized. TcBuster edited clones demonstrated higher antibody titer **(3A)** and copy number **(3B)** compared to clones generated by random integration. Pool titers (shown in Figure 2) were predictive of clone titers, with consistently higher titers in the clones generated from the TcBuster pool. The top TcBuster clone approached 1 g/L CD28 antibody titer by day 10 in culture, compared to < 100 mg/L for all random integration clones.

Genetic stability of CHO-K1 clones was assessed by culturing clones throughout 30 doublings (46 days). Clones were then cultured for 7 days in shaker flasks as previously described and samples were collected for analysis of antibody titer and copy number. Antibody titers of TcBuster clones at 0 and 30 doublings were higher than clones generated by random integration **(3C)**. Copy number of clones measured by digital PCR from samples collected at 0 and 30 doublings were also higher across all TcBuster clones **(3D)**. Antibody titer was consistent between the 0 and 30 doubling timepoints, with some fluctuation likely due to declining cell health after 7 days with no media change. After 30 doublings TcBuster clones maintained antibody production > 400 mg/L, outperforming the random integration clones throughout 46 days in culture.

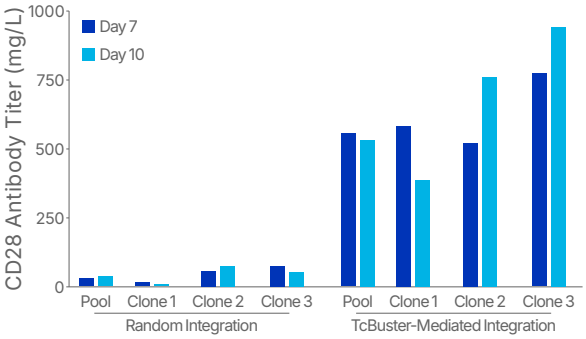


Figure 3A. Initial Antibody Titer of Clones

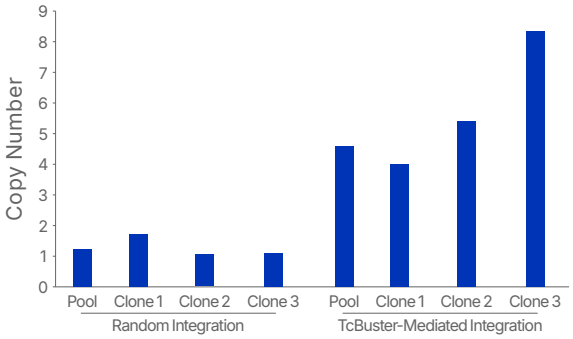


Figure 3B. Initial Copy Number of Clones

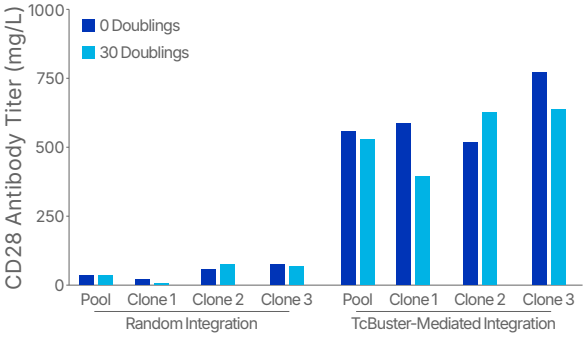


Figure 3C. Stability of Antibody Titer

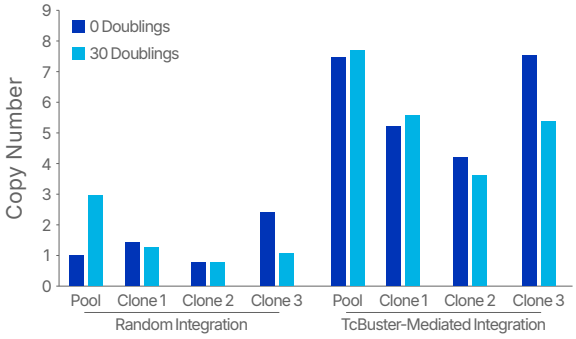


Figure 3D. Stability of Copy Number

Figure 3. TcBuster edited single cell clones have higher antibody titers and integrated copies than plasmid alone at 0 and 30 doublings.

Further Analysis of Top Performing Clones in a High-Throughput Bioreactor

Clone 3 was selected from both the random integration and TcBuster-mediated integration pool for an additional study using the Ambr 250 high-throughput bioreactor, which allowed for testing several culture conditions in parallel fed-batch bioreactor vessels. Clones were inoculated into reactors under four different reactor conditions for a total of 14 days. Reactor conditions included Dynamis or ActiPro media and a temperature maintained at 36.5 °C throughout the 14 days or decreased to 33 °C on day 5 **(4A)**. As seen in the shaker flasks, the TcBuster edited clone again far outperformed the random integration clone. Over the 14 days, the random integration clone produced < 1 g/L antibody under all conditions tested **(4B)**, while the TcBuster edited clone achieved approximately 5 g/L with conditions 1-3 and 12 g/L with condition 4 **(4C)**. A comparison of the day 14 antibody titer highlights the antibody production of the TcBuster clone compared to the random integration clone across all bioreactor conditions **(4D)**. Error bars show the standard deviation of replicate bioreactors (n=2).
The TcBuster clone culture resulted in antibody titer ranging from 8-24-fold higher than the random integration clone for each condition. This study further demonstrates the potential for TcBuster-mediated gene editing to aid in the efficient development of highly productive cell lines. With only one TcBuster clone and four conditions tested, there is potential for further optimization of antibody titer utilizing the Ambr 250.

Condition #	Media	Temperature (°C)
1	Dynamis	36.5
2	Dynamis	33
3	ActiPro	36.5
4	ActiPro	33

Figure 4A. Summary of Bioreactor Conditions

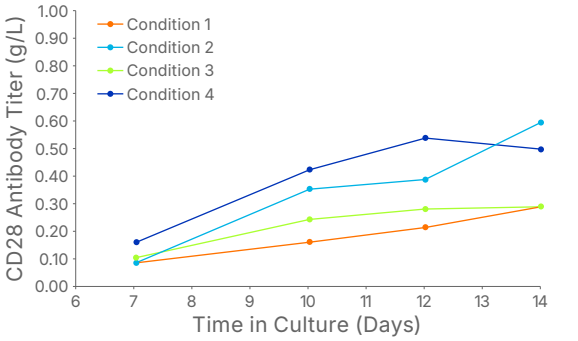


Figure 4B. Productivity of Random Integration Clone #3 (g/L)

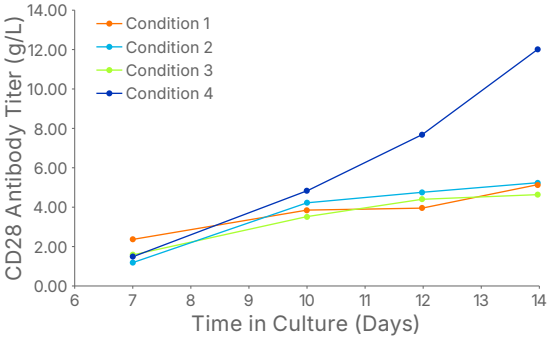


Figure 4C. Productivity of TcBuster-Mediated Integration Clone #3 (g/L)

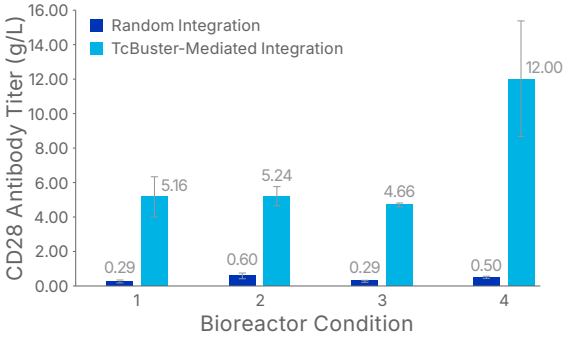


Figure 4D. Day 14 Antibody Titer

Figure 4. Top TcBuster edited single cell clone has high antibody titer ranging from 4.66-12.0 grams/L after 14 days in bioreactor culture.

Conclusion

The development of cell lines to produce biotherapeutics is both time- and labor-intensive, requiring the screening of hundreds of clones to identify top candidates from cell pools generated by random integration. In this study, the process of developing CHO-K1 cell lines through random integration or utilization of the TcBuster system was directly compared on a small scale. The TcBuster heterogeneous cell pool had 5-fold higher antibody titer than the random integration pool, which was reflected in the higher titer top clones isolated from each pool. Through minimal screening (< 20 clones per pool), the TcBuster pool resulted in top clones producing > 500 mg/L CD28 antibody titer in shaker flasks, while the random integration top clones produced < 100 mg/L. Running one clone each on a high-throughput bioreactor for 14 days resulted in a maximum of 12 g/L antibody produced from the TcBuster clone across four conditions tested, a 20-fold increase over the maximum titer achieved from the random integration clone.

Given the considerable time and effort required to single cell clone, expand, and analyze clones for bioprocessing cell line development, the use of TcBuster to increase gene integration allows for more efficient generation of high titer clones compared to random integration. Considering the extensive labor involved in developing cell lines, the ability to screen fewer candidates while still identifying high titer clones is a significant benefit of using the TcBuster system for cell line development. Additional optimization could include screening more clones and testing different media and culture conditions to further enhance antibody production.

References

1. Kheirandish, M.H., Rahmani, B., Zarei Jaliani, H. *et al.* (2023). Efficient site-specific integration in CHO-K1 cells using CRISPR/Cas9-modified donors. *Mol Biol Rep*: 50, 5889–5899. **https://doi.org/10.1007/s11033-023-08529-8**

2. Wang, B., Albanetti, T., Miro-Quesada, G. *et al.* (2018). High-throughput screening of antibody-expressing CHO clones using an automated shaken deep-well system. *Biotechnol Prog.* **https://doi.org/10.1002/btpr.2721**

3. Bio-Techne (2024). Lentiviral vs. Non-Viral Delivery for CAR-T Manufacturing: De-risking Cell Therapy Manufacturing with the TcBuster™ Non-Viral Transposon System. **https://www.bio-techne.com/resources/literature/lentiviral-transduction-non-viral-gene-delivery-car-t-manufacturing**

4. Clarke, H., Mayer-Bartschmid, A., Zheng, C. *et al.* (2024). When will we have a clone? An industry perspective on the typical CLD timeline. *Biotechnol Prog.* **https://doi.org/10.1002/btpr.3449**