

# Accelerating Cell Line and Process Development

Development of a high-producing cell line supported by a robust upstream process are critical early steps in the production of monoclonal antibodies (mAbs). Optimization of this upstream workflow is essential to deliver a sufficiently high titer to support clinical studies, and ultimately commercialization of the therapeutic. Historically, many of the steps used to identify clones and then scale the production process have been time-consuming and labor-intensive – slowing progress right from the starting gate.

This whitepaper describes the integration of technologies to more rapidly identify high-producing cell lines, strategies to optimize media feeds and a robust bioreactor scale-up approach to create a consistent and reliable solution for upstream bioprocess development.

## Improving the Efficiency of Cell Line Development

A low process titer may render an antibody too costly to manufacture and unsuitable for continued investment. Unfortunately, the quest to find the best-producing clone has been compared to looking for a needle in a haystack.

The CHOZN® & UCOE® Combined Platform enables more rapid and efficient selection of high-producing cell lines, accelerating upstream processes and saving time and money.

This complete toolkit contains all the materials needed to advance from the protein sequence to a production cell line. It includes the CHOZN® GS<sup>-/-</sup> cell line, an expression plasmid containing ubiquitous chromatin opening element (UCOE®) technology, a set of cell culture media and feeds that can be used through production scale, along with protocols and documentation for regulatory.

## CHOZN® GS<sup>-/-</sup> Cell Line

The CHOZN® GS<sup>-/-</sup> cell line, introduced in 2011, has both alleles of the glutamine synthetase gene deleted. To generate a cell line producing the gene of interest (GOI), the CHOZN® GS<sup>-/-</sup> is transfected with a plasmid containing the glutamine synthetase gene and the GOI. Absence of glutamine in the culture medium creates selective pressure on the cells which have integrated the plasmid, allowing them to survive.

## UCOE® Technology

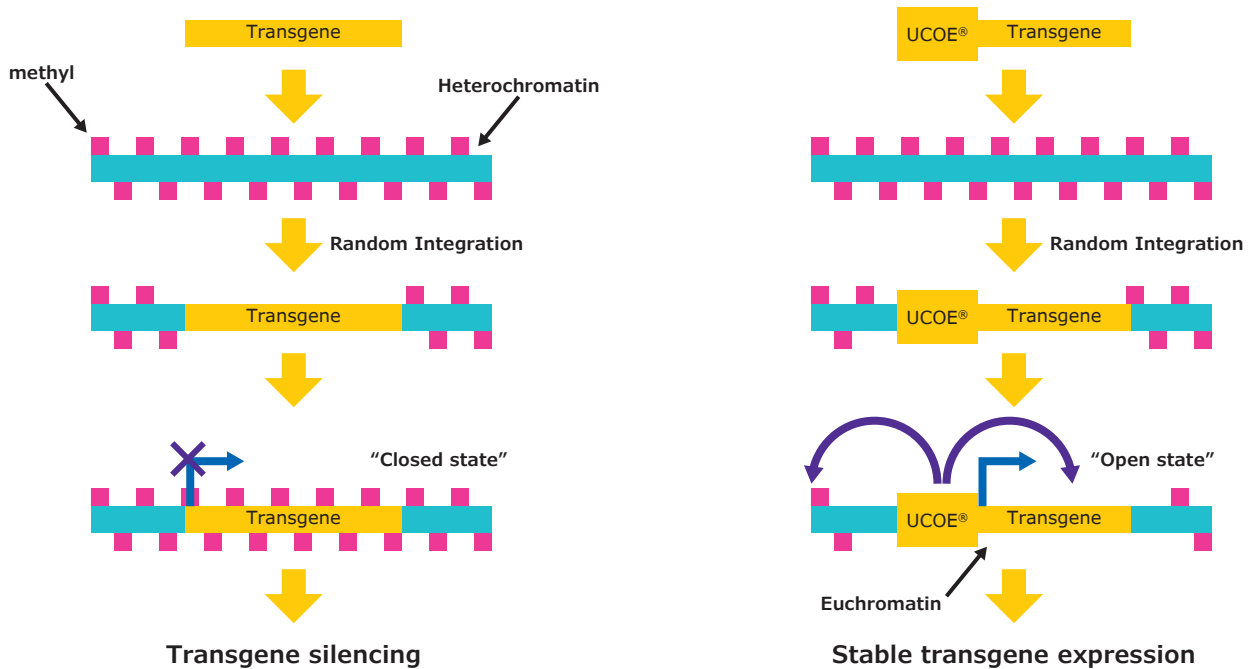
The cell line development process relies on random integration of the expression vector into the correct location of the host cell genome in order to permit survival during the selection process. Of the cells that survive the selection process, a small number express a sufficient titer of the recombinant protein to serve as a production-scale clone.

Finding these precious clones requires screening of thousands of candidates, done either manually or with use of automation to perform rounds of single cell seeding, feeding and clone selection. While automation can reduce time requirements, these systems are costly and require dedicated personnel.

An alternative to these approaches is to increase the number of high-performing clones – in other words, seed more needles into the haystack. This can be accomplished by combining UCOE® expression technology with the CHOZN® GS<sup>-/-</sup> cell line.

The specific location of the genome into which the transgene integrates, and whether that area consists of heterochromatin or euchromatin, plays an important role in expression of the transgene. Most of the genome is organized into tightly wound, transcriptionally inactive heterochromatin. In contrast, a portion of the DNA is in the open, transcriptionally active form called euchromatin. During random integration, if the transgene integrates into the heterochromatin, it becomes rapidly methylated by host cell enzymes. Following methylation, the DNA is tightly wound into histones and is not capable of supporting transcription.

## Ubiquitous Chromatin Opening Elements (UCOE®) Technology Increases Hit Rate

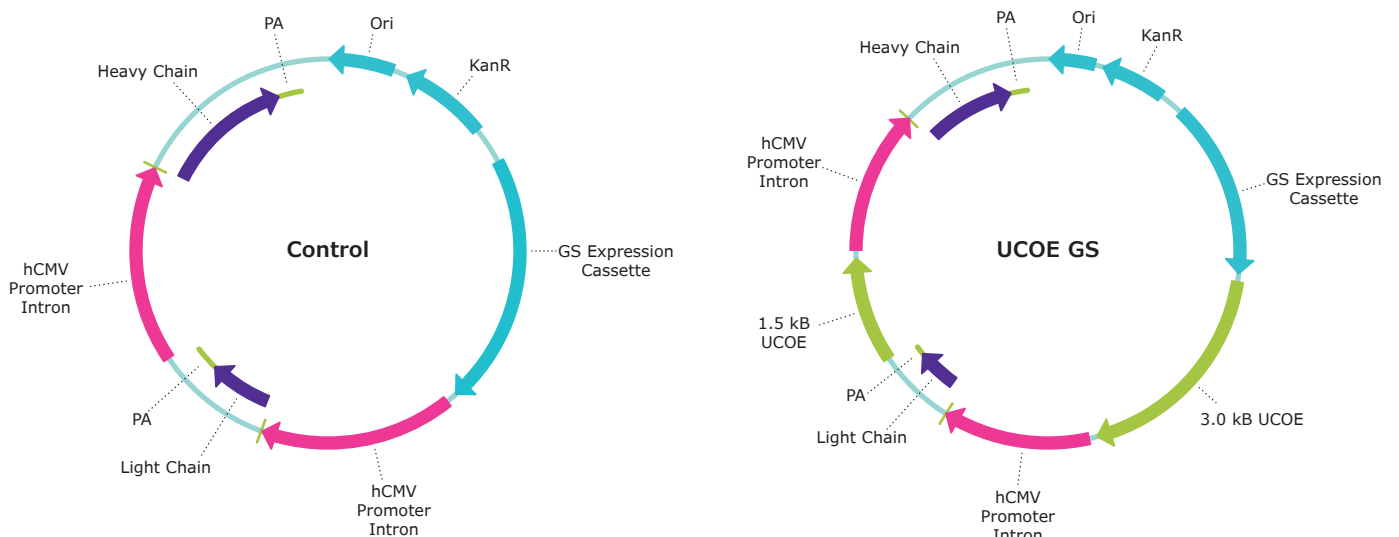


**Figure 1.** When a transgene is linked to UCOE®, methylation of the region is prevented and the DNA remains open and capable of transcription.

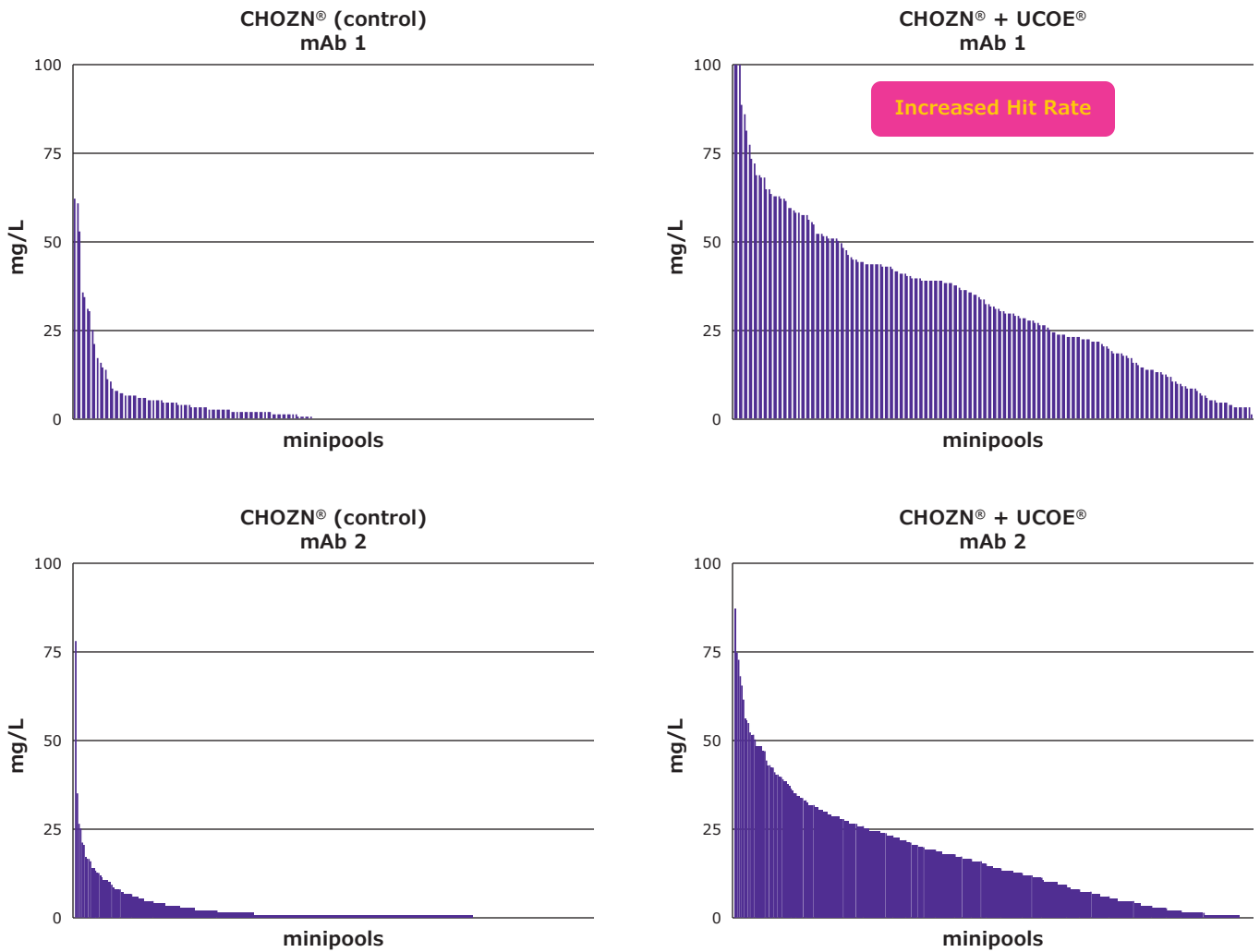
A UCOE® element is a segment of DNA that derives from the 5' control region of the promoters of essential housekeeping genes. This region has evolved to prevent the silencing of linked genes. When the transgene is linked to a UCOE®, methylation is prevented and the DNA remains open and capable of transcription (Figure 1). This approach to increase hit rates during cell line development has been used for more than 15 years and has been incorporated into the expression vector included in the CHOZN® & UCOE® Combined platform (Figure 2).

The vector contains a light-chain expression cassette with an hCMV promoter intron, a heavy-chain expression cassette, a glutamine synthetase cassette, a bacterial origin and selection marker. The expression construct includes two UCOE® elements; one is located upstream of the light-chain expression cassette and the other is located between the heavy-chain and light-chain expression cassettes.

## UCOE® Added to Standard CHOZN® Expression Vector



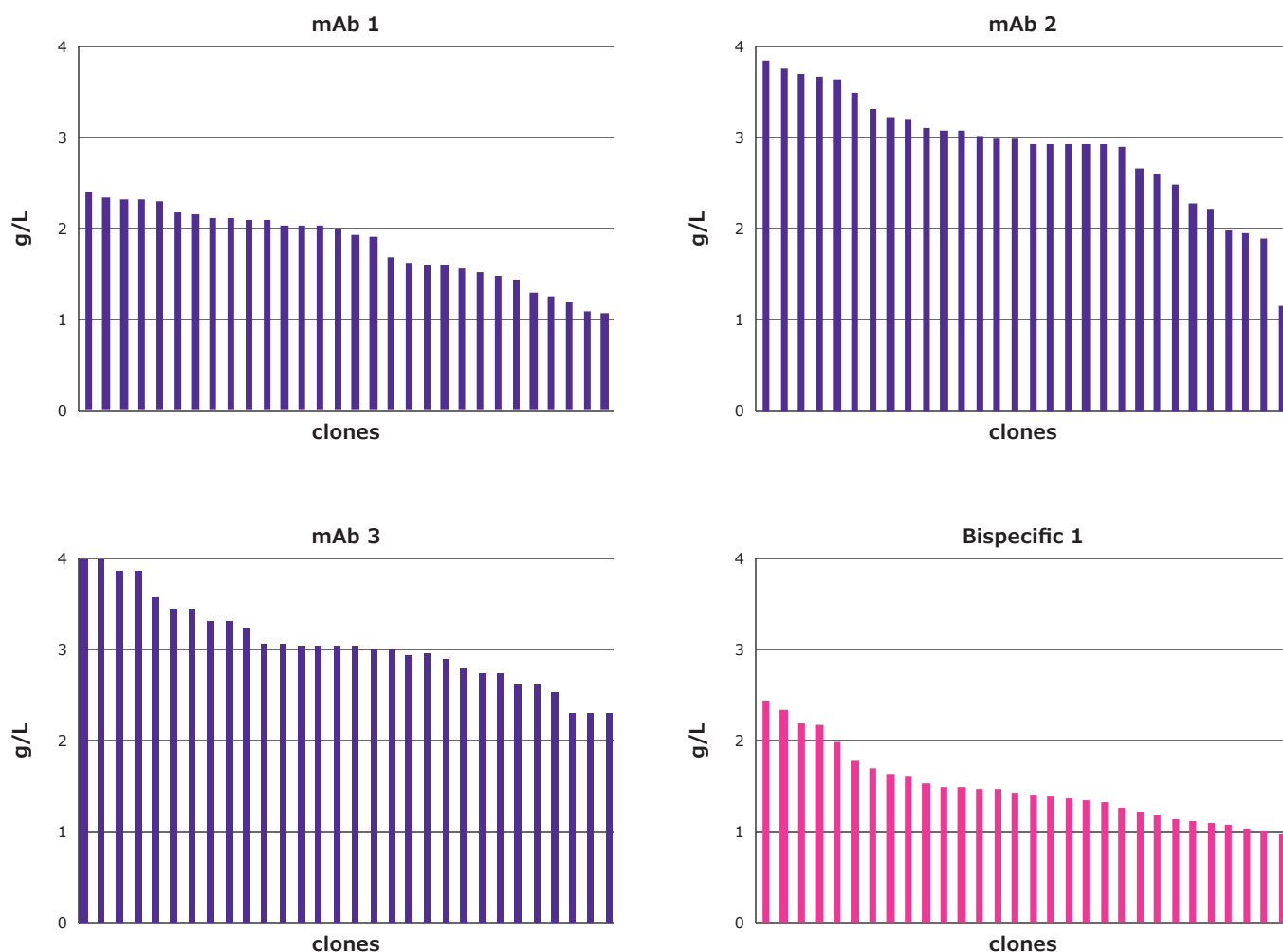
**Figure 2.** Standard CHOZN® expression vector (A) and vector with addition of UCOE®.



**Figure 3.** Incorporation of the UCOE® expression vector increased the number of high-expressing minipools.

To demonstrate the ability of the CHOZN® & UCOE® combined platform to increase the number of high-expressing clones, cells were transfected and following a short recovery period, thousands were plated into 96-well plates and cultured in the absence of glutamine for selection. The resulting minipools were then screened for titer.

Figure 3 shows the initial screening titers for the UCOE® control minipools in two cell line development studies. Each line represents the titer of a separate minipool, evaluated in a seven-day batch assay on 96 well plates. There was a striking difference between the average titers of the minipools.

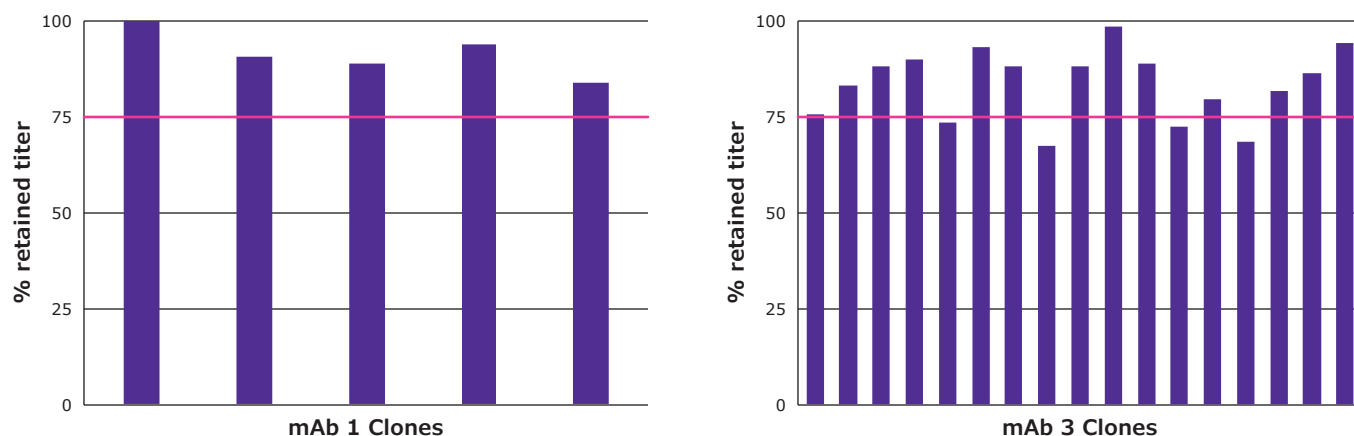


**Figure 4.** Titers for the top thirty clones from three mAb and one bispecific cell-line development projects.

With this increase in the hit rate, it was hypothesized that it would be easier to isolate high-expressing clones. The top producing UCOE® minipools were seeded for single-cell cloning and those top performing clones were subjected to fed-batch assays. Titers for the top thirty clones from three mAbs and one bispecific cell-line development projects are depicted in Figure 4. These results demonstrate that the CHOZN® & UCOE® combined platform can be used to generate high-performance clones.

The top clones from mAb #1 and #3 were subjected to an expression-stability test. Recombinant clones were passaged for at least 45 days; early-passage and late-passage cells were subjected to a fed-batch assay to determine the percentage of retained titer.

As shown in Figure 5A, each of the five clones of mAb #1 retained at least 75 percent titer after 90 days in culture. Seventeen expressing clones from mAb #3 were subjected to a 45-day expression-stability experiment; 13 of these clones maintained at least 75 percent of the early-passage expression (Figure 5B). This study further supports that recombinant CHOZN® & UCOE® clones exhibit expression stability.



**Figure 5.** CHOZN®-UCOE® clones exhibit stable recombinant expression.

## Accelerating Upstream Process Development

The high-producing clones generated by the CHOZN® cell line development platform and UCOE® technology were then subjected to a feed screening study and scale-up with Mobius® bioreactors to rapidly advance from bench to pilot scale.

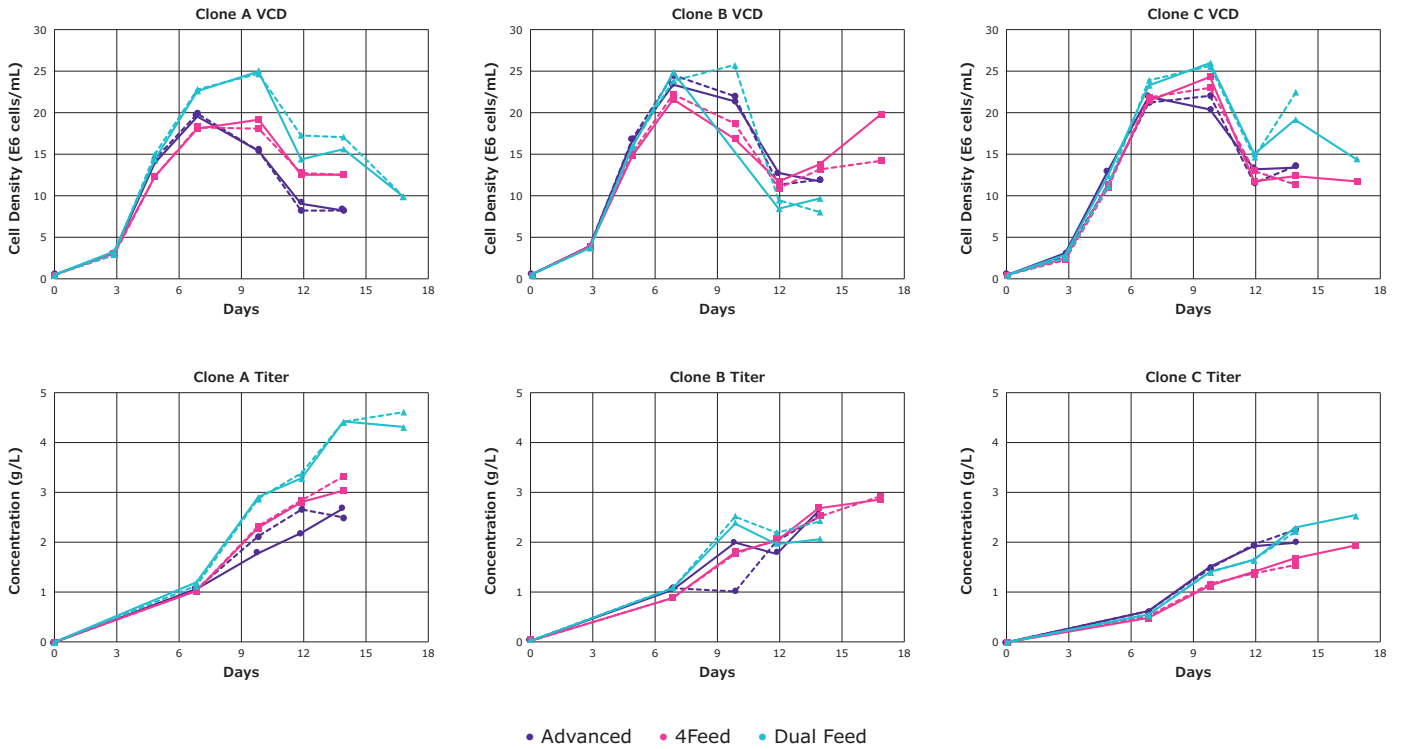
As a first step, performance of fed-batch cultures of top-producing mAb #1 clones was evaluated using a simple feed screen study of EX-CELL® Advanced CHO Feed 1 and Cellvento® 4Feed COMP (Table 1). Fed batch cultures were

grown in spin tubes using EX-CELL® Advanced CHO fed-batch medium. Data from preliminary studies suggested that a combination of the two feeds improved process performance (data not shown). To evaluate the combination feed, an extra set of tubes for each clone was run, wherein each feed was added in equal parts using the regimen developed for the EX-CELL Advanced Feed platform. Glucose was added as needed, based on consumption, with the target concentration increased as the cell density increased.

**Table 1. Summary of feeds evaluated during process development.**

Feed	Day 3	Day 5	Day 7	Day 10	Day 12	Total
Adv	5%	5%	10%	10%	0%	30%
4Feed	3%	3%	6%	6%	0%	18%
Adv + 4Feed	2.5%, 2.5%	2.5%, 2.5%	5%, 5%	5%, 5%	0%	30%
Glucose	6g/L	6g/L	9g/L	9g/L	6g/L	N/A

- Final stage of clone screen for mAb 1
  - CHOZN-GS®+UCOE®
  - Fed-batch cultures in spin-tubes
  - EX-CELL® Advanced CHO Fed-batch Medium
- Feeds evaluated:
  - EX-CELL® Advanced CHO Feed 1 (without glucose)
  - Cellvento® 4Feed
  - Advanced and 4Feed in equal parts
  - Fed every 2-3 days starting on day 3

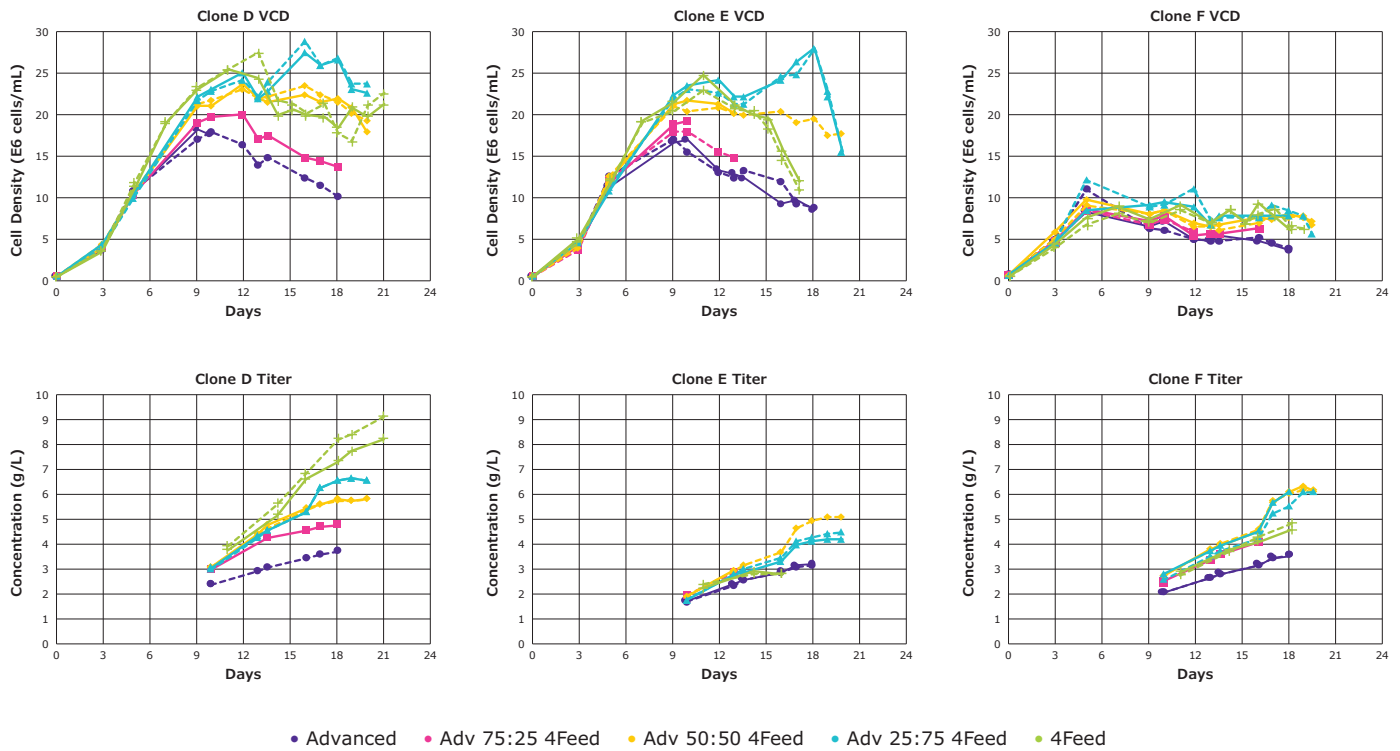


**Figure 6.** Comparison of three feeds on cell density and titer of three different clones producing mAb #1.

As shown in Figure 6, the different feeds led to differences in cell density and titer for the three different clones; duplicate cultures are represented by the same color, with one solid line and one dashed line.

For clone A, an increased peak cell density and corresponding increase in final titer to approximately 4.5g/L was observed when using the combination of the two feeds, as compared to either the Advanced feed or 4Feed individually. In contrast, the impact of the different feeds on clones B and C was minimal.

While the performance of the dual feed was beneficial in some of the clones tested, it is less desirable to have a platform process with two addition steps at each feed. Fortunately, these two particular feeds can be combined and fed as a single-unit operation. In order to do so, each feed is hydrated individually, according to the applicable instructions. Once the hydration is completed, the feeds can be mixed together in the desired ratio; this can be done either prior to the sterile filtration step or after, if combined aseptically.



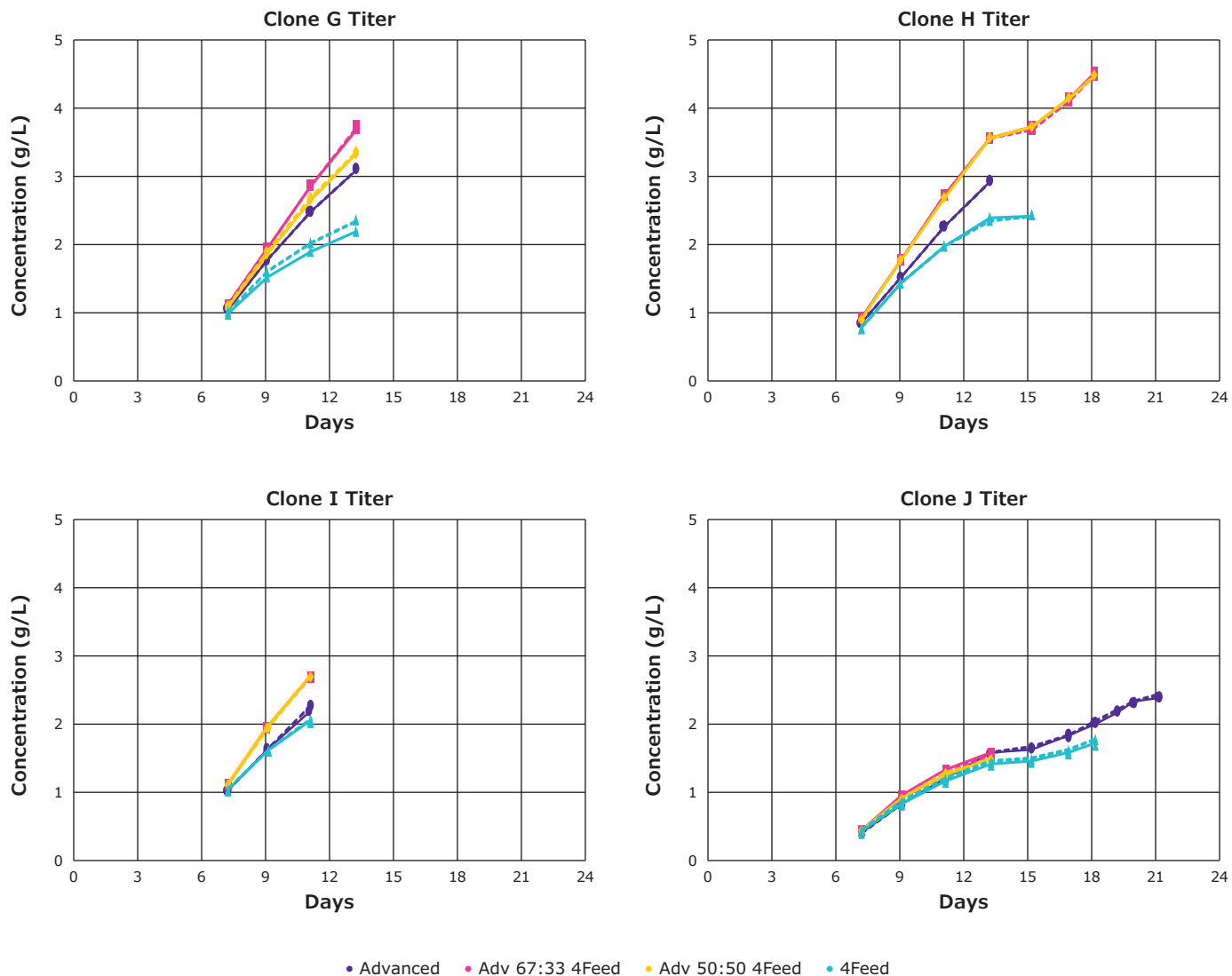
**Figure 7.** Comparison of five feeds on cell density and titer of three different clones producing mAb #3.

While the combination of the two feeds improved the performance of some of the clones tested, the impact varied from clone to clone. Given this finding, the use of blend ratios other than 50-50 were investigated to determine if different blends were preferred by high-performing clones producing mAb #3. In this study, two blends were evaluated in addition to the 50-50 blend:

- 75% Advanced and 25% 4Feed
- 25% Advanced and 75% 4Feed

From the results shown in Figure 7, it is evident that the preferred feed or blend varied between clones. Both clones E and F preferred a blend to either of the individual feeds, while clone D preferred the 4Feed only compared to any of the blends tested. While clone E demonstrated a clear preference for the 50-50 blend, clone F showed equivalent results with the 50-50 blend and the 25% Advanced and 75% 4Feed blend.

Interestingly, the mechanism by which the performance improves can vary. In some cases, the preferred feed results in an increase in biomass, which subsequently translates to an increase in volumetric titer, as seen in clones D and E. While in other cases, there was no discernible increase in cell density, but a significant increase in volumetric titer was observed, indicating increase in cell-specific productivity, as seen in clone F.



**Figure 8.** Comparison of four feeds on titer of four different clones producing Bispecific 1.

The previous study demonstrated that through a relatively simple feed screening, the process performance for standard mAb production can be improved. A similar study was conducted with a more difficult-to-express bispecific molecule produced in the CHOZN® cell line using UCOE® to determine if the feed screen can similarly improve performance of that process. In this study, both the Advanced feed and Cellvento 4Feed individually were evaluated, as well as a 50-50 mixture and a 67% Advanced and 33% 4Feed blend. Cultures were run in spin tubes using EX-CELL® Advanced fed-batch basal medium.

As indicated by the volumetric titer results for the top four clones tested, a varied response to the use of the blends as compared with the individual feeds was observed (Figure 8).

While the blends appear to be as good or better than the individual feeds, the degree of improvement was variable among clones. In both clone I and H, the performance of the two blends appeared to be equal. While in clone G, the 67% Advanced and 33% 4Feed blend outperformed the 50-50 blend. In clone J, the Advanced-only and 4Feed-only conditions performed better overall than the blends; this was due solely to the slightly higher viability allowing the culture to extend for a longer period of time.



Once the feed strategy is optimized, the process can be applied to bioreactor production. This process can be accelerated by use of a robust platform process that consistently exhibits high performance with regards to cell growth and volumetric titer. Use of this platform process enables rapid evaluation of top-performing clones to determine which single clone will be selected to move forward.

The foundation of this concept is that it is simpler and faster to pick the clone that fits a robust process than to redesign the entire process for a specific clone for every project.

By selecting a clone that fits the process, the bioreactor development timeline can be reduced by months. Instead of designing a process from scratch, minor adjustments can be made, if needed, to the existing process to reach the desired performance level.

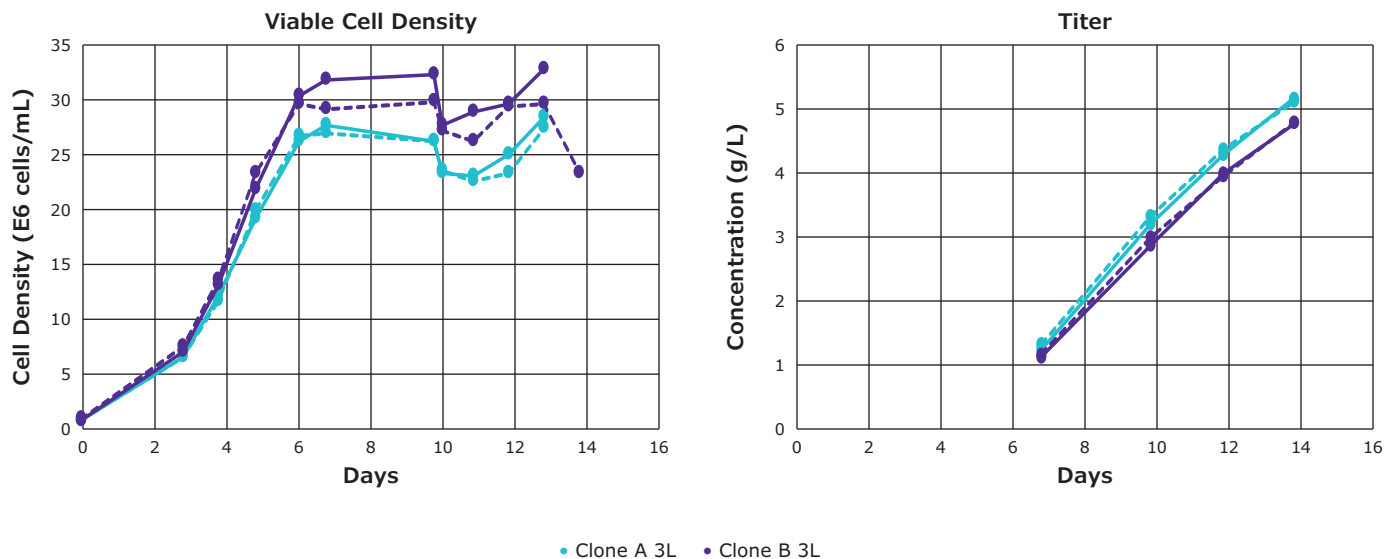
This platform process has been designed for consistency and robustness, but also prioritizes speed. Process parameters are set such that they are translatable across scales, which allows rapid scaling from bench to pilot and reduction in possible process development bottlenecks. This approach enables more rapid technology transfer and generation of material to support toxicology studies or phase one clinical trials. While this process is both robust and consistent, it is designed in such a way that it works well for most cell lines and clones but

can be optimized through more thorough development. This will become a higher priority than speed if and when a project advances to phase two and beyond.

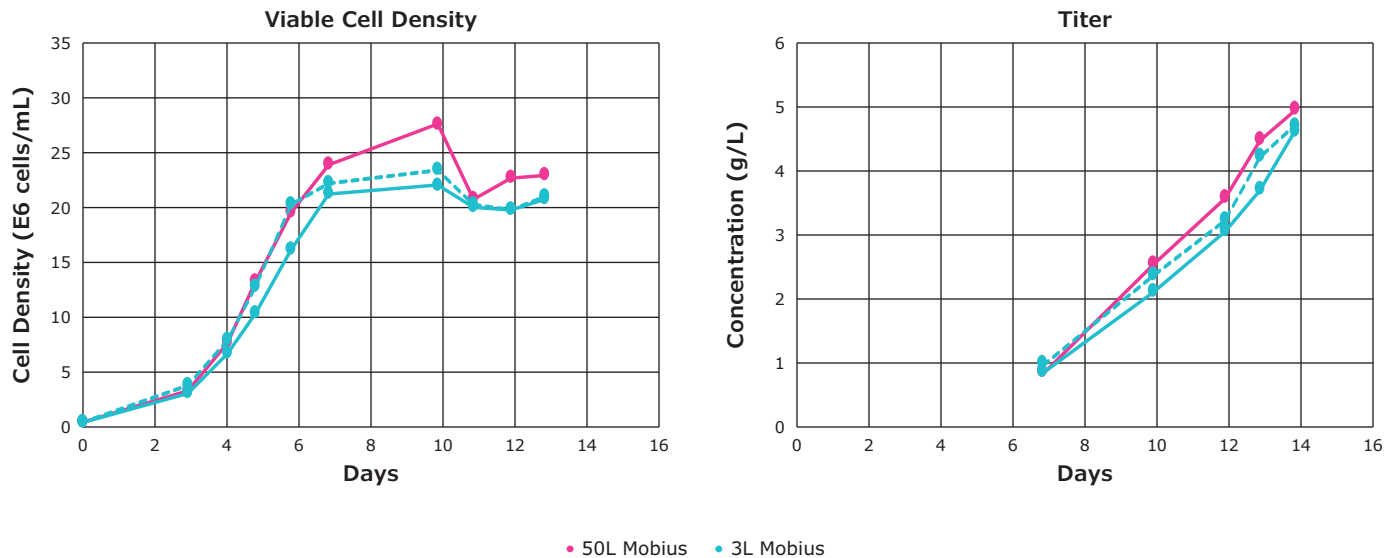
Table 2 summarizes the current platform process for the CHOZN® GS<sup>-/-</sup> cell line which works well for the majority of clones tested. The time and effort invested on additional optimization will be dictated by the stage of the project and the project needs with regards to volumetric titer and product quality. One parameter that can be optimized for a cell line and clone is the feed strategy. While the feed amount and schedule shown in Table 2 work well for the platform, this can vary depending on the growth profile and nutrient consumption of a specific clone. One method that has worked well is use of a viable cell density trigger to determine feed percentage and timing.

**Table 2. Process development bioreactor platform optimized for CHOZN® GS<sup>-/-</sup> clones.**

Media and Feed		Bioreactor Conditions	
Growth media	EX-CELL® CD Fusion	Agitation	~ 14 W/m <sup>3</sup>
Production media	EX-CELL® Advanced Fed Batch	DO setpoint	30%
Feed composition	EX-CELL® Advanced CHO Feed 1	Sparging strategy	O <sub>2</sub> through open pipe
	Cellvento® 4Feed COMP	pH set point/range	6.9 (+ 0.10 / - 0.15)
	Blend of Advanced Feed 1 and 4Feed	pH control	CO <sub>2</sub> and NaCO <sub>3</sub>
Feed amount and schedule	5% on days 3, 5, and 12 7.5% on days 7 and 10 Glucose as needed	Confirmation of conditions in larger bioreactors	



**Figure 9.** Cell density and titers for two clones in 3L Mobius® bioreactors.



**Figure 10.** Cell density and titer for top performing clone in 3L and 50L Mobius bioreactors.

This platform was used in the following bioreactor studies; duplicate cultures are represented by the same color, with one solid line and one dashed line. Figure 9 summarizes evaluation of two top mAb #1-producing clones and the bioreactor platform at the 3-liter scale. While clone B was able to achieve a slightly higher peak cell density, clone A achieved a slightly higher volumetric titer of approximately of 5 g/L, and was therefore selected as the top clone for this project. However, clone B was retained as a viable backup, should clone A have any unforeseen issues, for example, with product quality.

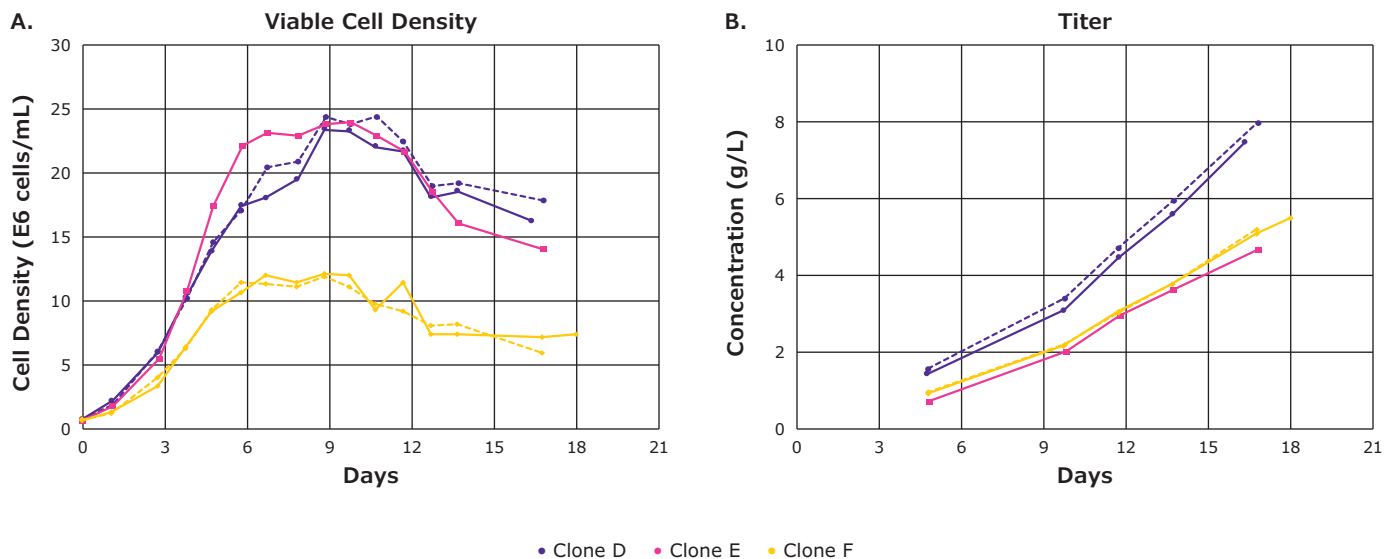
Performance of clone A was subsequently evaluated at the 50-liter scale (Figure 10). Thanks to the robustness of the process, a comparable performance was achieved between the 3L and 50L scales in the first study. These results clearly demonstrate the benefit of a robust process to streamline the workflow from clone screening to scale-up.



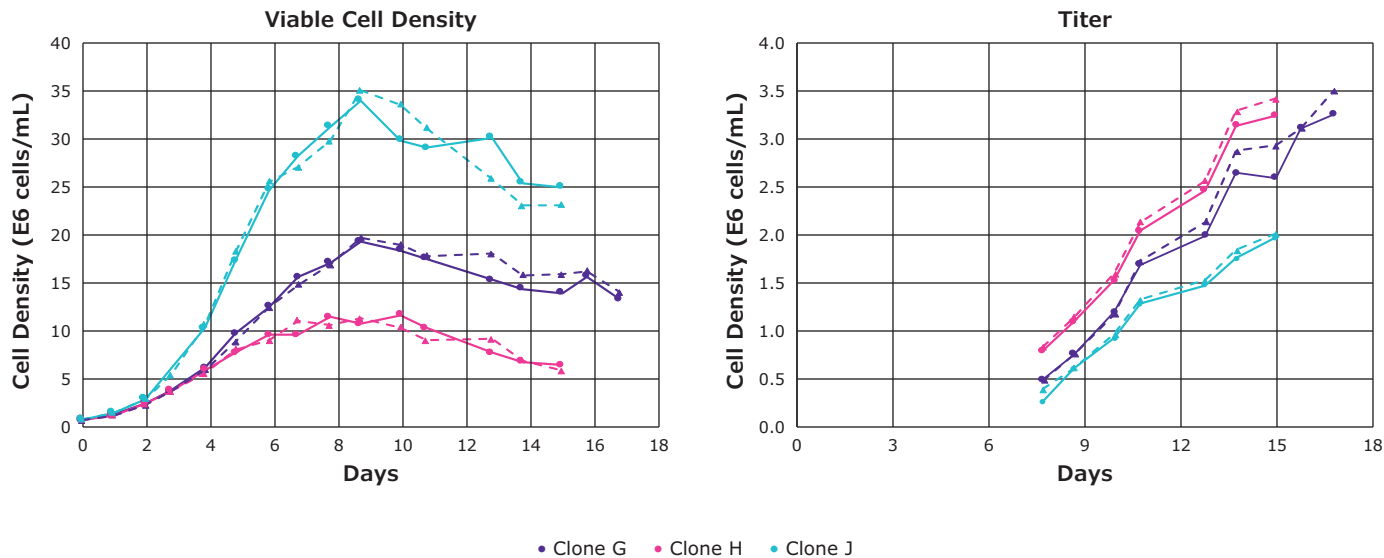
Mobius® 3L



Mobius® 50L



**Figure 11.** Evaluation of top-performing clones in the bioreactor platform process in terms of cell density (A) and titer (B).



**Figure 12.** Bioreactor evaluation for a more difficult-to-express bispecific.

Figure 11 provides a second example of the rapid evaluation of top-performing clones from mAb #3 in the bioreactor platform process. The fed-batch process performed consistently in bioreactors as compared to the spin tubes with comparable peak cell densities and volumetric titers for each clone. Figure 11A shows that both clones D and E achieved a peak cell density of close to  $25 \times 10^6$  cells/ml, while clone F peaked closer to  $12 \times 10^6$  cells/ml. Figure 11B shows that clone D achieved a titer of about 8 g/L, while E and F were between 4-5 g/L. Given these results, clone D was selected to advance, due to its high titer. Clone F was identified as having potential for profusion applications, due to its decent titer but low peak cell density, which translates into a high cell-specific productivity level.

Figure 12 summarizes results of the bioreactor evaluation for a more difficult-to-express bispecific. The top three clones identified following the previous feed screen study were subjected to the platform process for fed-batch bioreactors. Figure 12 shows vastly different peak cell densities with regards to the three clones; clone J reached  $35 \times 10^6$  cell/ml, clone H reached  $20 \times 10^6$  cell/ml, and clone G reached  $10 \times 10^6$  cell/ml. The ranking order is reversed, however, for the volumetric titer values (Figure 12B); clones G and H reached between 3-3.5 g/L and clone J reached 2 g/L.

## Conclusion

The integration of the CHOZN® GS<sup>-/-</sup> platform, UCOE® expression technology, feed screens and a robust bioreactor scale-up approach delivers a consistent and robust solution for upstream bioprocessing needs.

Over many years of successful use in industry, the CHOZN® GS<sup>-/-</sup> cell line has shown to be robust and capable of producing high titers in fed-batch production. Combining this cell line with UCOE® technology can increase the hit rate of high-producing clones. Implementing a feed-screen step in the development strategy can significantly improve the performance of fed-batch processes; many cell lines and clones have demonstrated increased cell densities and volumetric titer with the use of feed blends. However, it is important to determine what is best for a particular clone as some prefer EX-CELL® Advanced feed one or Cellvento® 4Feed only, while others prefer different blend ratios.

Use of a series of scalable bioreactors, such as Mobius bioreactors, will significantly reduce process development timelines.

Finally, development timelines can be significantly reduced by leveraging a robust platform process, which allows for rapid evaluation of top clones in bioreactors and rapid scale up from bench to pilot scale. It should be noted, however, that this approach prioritizes speed over perfection. It can help get a process ready for tech transfer sooner, which translates into generating material for toxicology studies and phase-one trials earlier. However, there are aspects that can be optimized to improve process performance further, which is important when locking in a process for phase two and beyond.

## To place an order or receive technical assistance

In Europe, please call Customer Service:

France: 0825 045 645	Spain: 901 516 645 Option 1
Germany: 069 86798021	Switzerland: 0848 645 645
Italy: 848 845 645	United Kingdom: 0870 900 4645

For other countries across Europe, please call: +44 (0) 115 943 0840

Or visit: [MerckMillipore.com/offices](https://www.MerckMillipore.com/offices)

For Technical Service visit: [MerckMillipore.com/techservice](https://www.MerckMillipore.com/techservice)

[MerckMillipore.com](https://www.MerckMillipore.com)

Merck KGaA  
Frankfurter Strasse 250  
64293 Darmstadt, Germany

