Accelerating Cell Line Development for Complex Proteins

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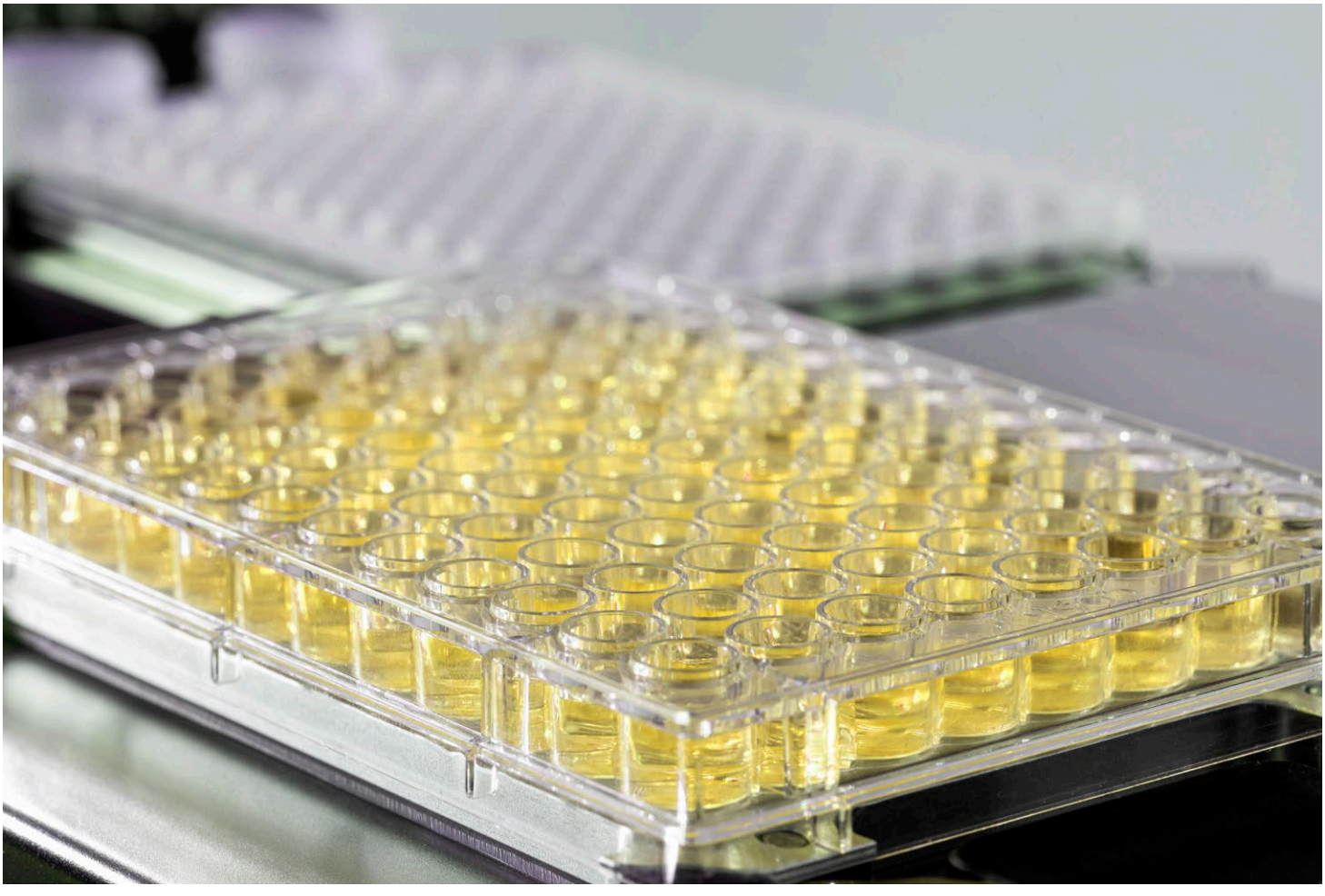
Simplifying Progress

SARTORIUS

Introduction

As the industry advances toward more complex modalities—such as bi- and multispecifics, fusion proteins, and other non-classical antibodies—the limitations of traditional screening approaches to clone selection have become increasingly apparent. Achieving robust expression, stability, and manufacturability for these challenging molecules requires a flexible, toolbox-based strategy that integrates diverse technologies, data-driven insights, and process expertise across the entire workflow.

This white paper first describes our CHO cell line development workflow, which integrates automation, predictive modeling, machine learning, and very early productivity assessment to enable the rapid identification of high-performing clones. We then discuss our flexible toolbox, which can be applied to provide tailored support for the production of complex and difficult-to-express proteins. Using this integrated workflow, we reduce development timelines, improve product yield and quality, and de-risk scale-up. Finally, drawing on real-world results, we illustrate improvements in expression and process robustness across a range of complex biologics.



At Sartorius, these data-driven approaches are embedded within a toolbox designed to address the diverse challenges of multispecifics and difficult-to-express modalities. This includes multiple vector formats tailored to complex molecular architectures, optimized transfection processes, and an optional mini- or large-pool screening before single-cell cloning to ensure early assessment of expression and product quality. Targeted media and process optimization, as well as high-inoculation fed-batch (HIFB) and perfusion workflows, further enhance titer, improve product stability, and streamline scale-up.

Drawing on recent case studies and technological advances, this white paper highlights how integrating automation, predictive modeling based on multi-parameter analytics, machine learning, and very early productivity analytics enable the prompt identification of high-performing clones. When combined with media and process optimization as well as process intensification approaches, performance is further enhanced. Collectively, these tools reduce development timelines, improve product yield and quality, and de-risk scale-up. We also outline practical steps for implementing these predictive and performance-enhancing workflows and examine how this integrated approach is already improving product expression and process robustness.

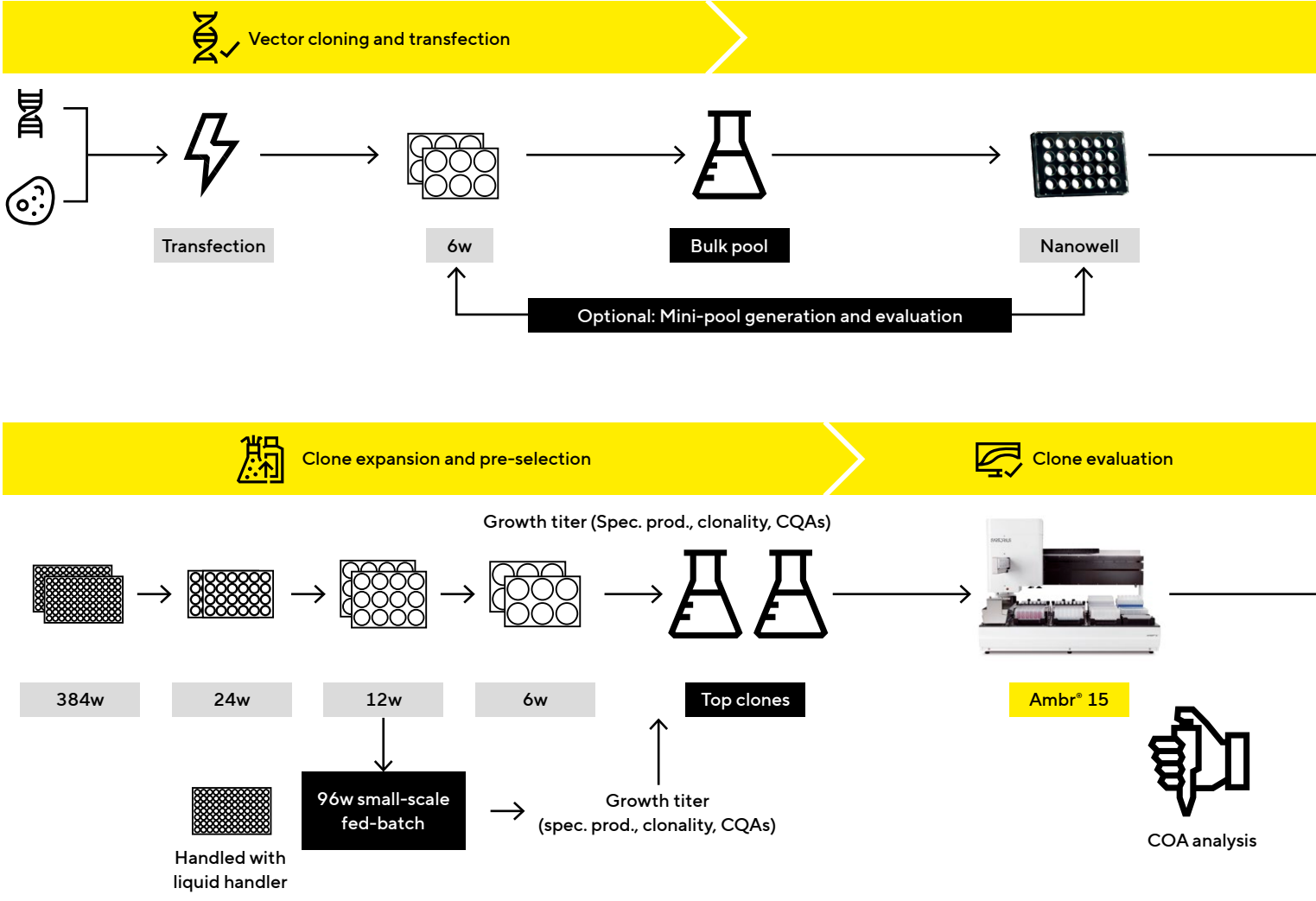
Sartorius CHO Cell Line Development Platform

Our CHO Cell Line Development Platform provides a robust foundation for cell line development, centered on:

- Genetically engineered CHO host cell line: Optimized for recombinant therapeutic protein production. Fully documented, including comprehensive cell line history and testing compliance according to regulatory standards
- Optimized expression vectors: Mono- and bi-cistronic vectors designed with full freedom-to-operate
- 4Cell® SmartCHO Media System: Formulated to extract the optimal performance of the cell line

An overview of our standard cell line development workflow is shown in Figure 1. The process begins with vector cloning and transfection of the client's gene of interest into our optimized CHO expression vector, followed by introduction of this plasmid into our CHO host. The generated large pool is then subjected to automated single-cell cloning. During this process, an image-based verification is used to ensure monoclonality.

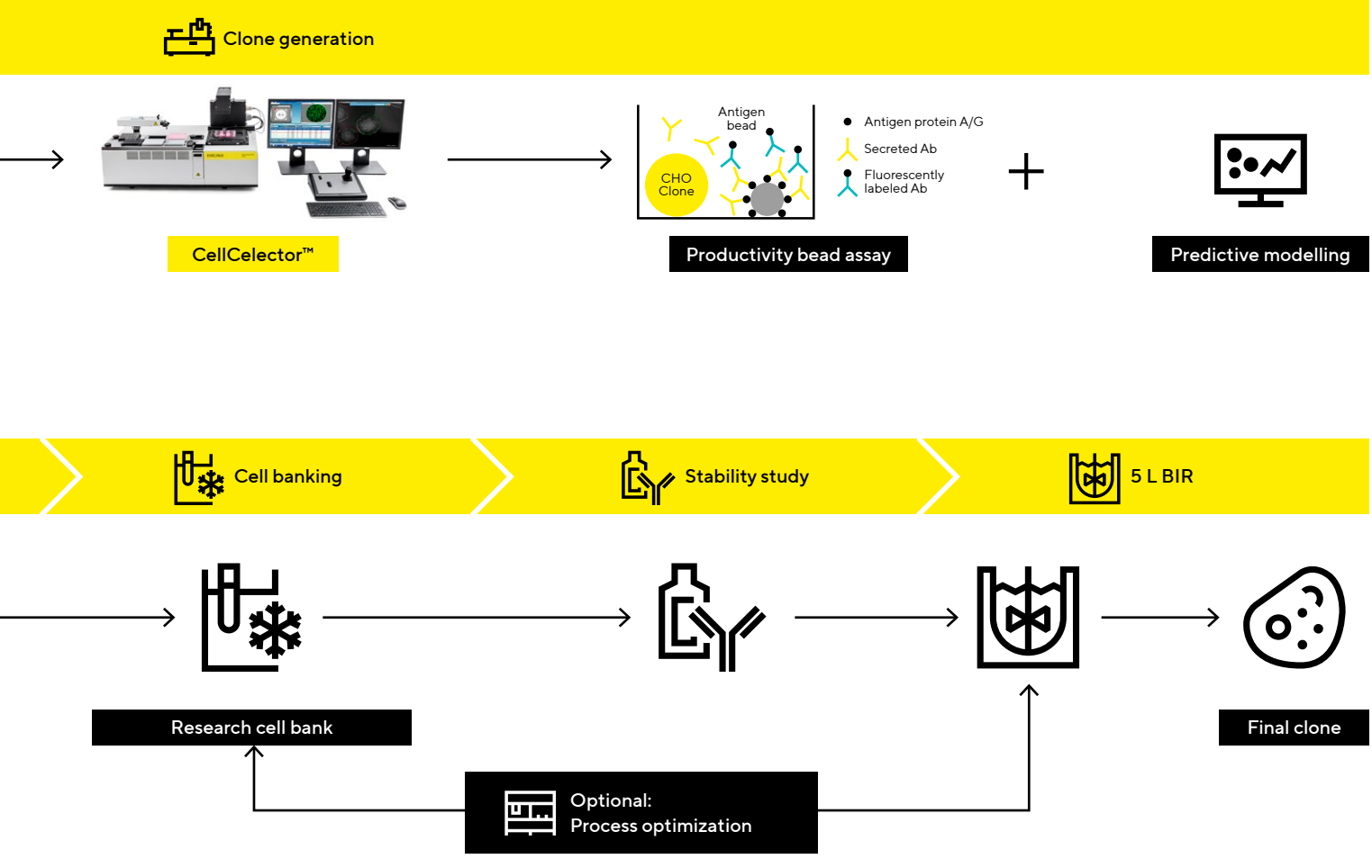
Figure 1: Overview of the Sartorius CHO Cell Line Development Platform



In addition, a productivity-based assay and predictive modeling using artificial intelligence (AI) | ML are applied to identify high performers. Next, during clone expansion, a high-throughput screening in small-scale fed-batch mode is performed to identify the top clones, which are further evaluated in Ambr® 15 | Ambr® 250 fed-batch systems for productivity, growth, and quality.

After just 9 weeks, the top 4 clones are established as a research cell bank (RCB). These stable, high-yielding clones are then advanced through stability testing and a 5 L bioreactor confirmation run to validate scalability. At this stage, an optional process optimization outside the main development workflow can be initiated. Throughout the program, Sartorius provides expert guidance, regulatory-compliant documentation, and flexible options for process optimization and tech transfer, enabling rapid and confident progression toward IND submission.

In the following sections, we explore the key stages and technologies that define our CHO cell line development workflow.

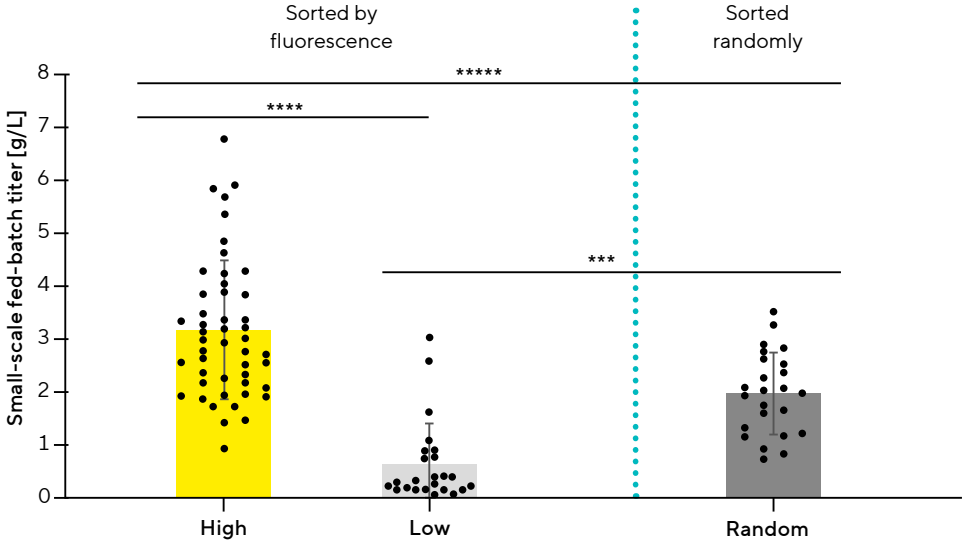


Single-cell cloning with CellCelector

The high-throughput automation capabilities of the CellCelector™ platform enable fast and robust single-cell cloning to isolate and select a monoclonal cell line from established stable pools. The cells are inoculated and cultured in a 24-well plate, with each well consisting of 4,300 nanowells. Automated image-based analysis is used to determine monoclonality.

The supportive nanowell environment also enables 40 – 50% higher clone outgrowth and recovery compared to traditional methodologies, such as flow cytometry, supporting rapid progression to 384-well formats for further clone expansion just four days after single-cell cloning. Our proprietary Protein A bead productivity assay leverages the fluorescence capabilities of the CellCelector to reliably identify high-producing clones (Figure 2).

Figure 2: Evaluating the selection stringency of the bead productivity assay. Titers of clones identified as high producers (yellow bar) in the productivity bead assay showed significantly higher titer in fed-batch evaluation compared to randomly selected clones (dark grey)



Smart selection through predictive modeling

To further improve clone selection, we combine the fluorescence productivity assay and other data captured by the CellCelector with predictive modeling. During single-cell cloning of various products, we collected a range of different cell and fluorescence parameters. We leveraged this data to create and train an AI and ML data model, which we now utilize during client programs.

The model uses three AI | ML algorithms: principal component analysis (PCA) classification, an artificial neural network classifier, and a random forest classifier to differentiate high- and low-producing clones. All three algorithms then process the data through SIMCA® software with a one-button interface, which provides a ranking of clones determined to be high producers. The model is continually retrained to improve predictive accuracy.

Figure 3: Comparison of fed-batch titers for two products when clone evaluation was based on the productivity assay alone vs. the productivity assay combined with predictive modeling

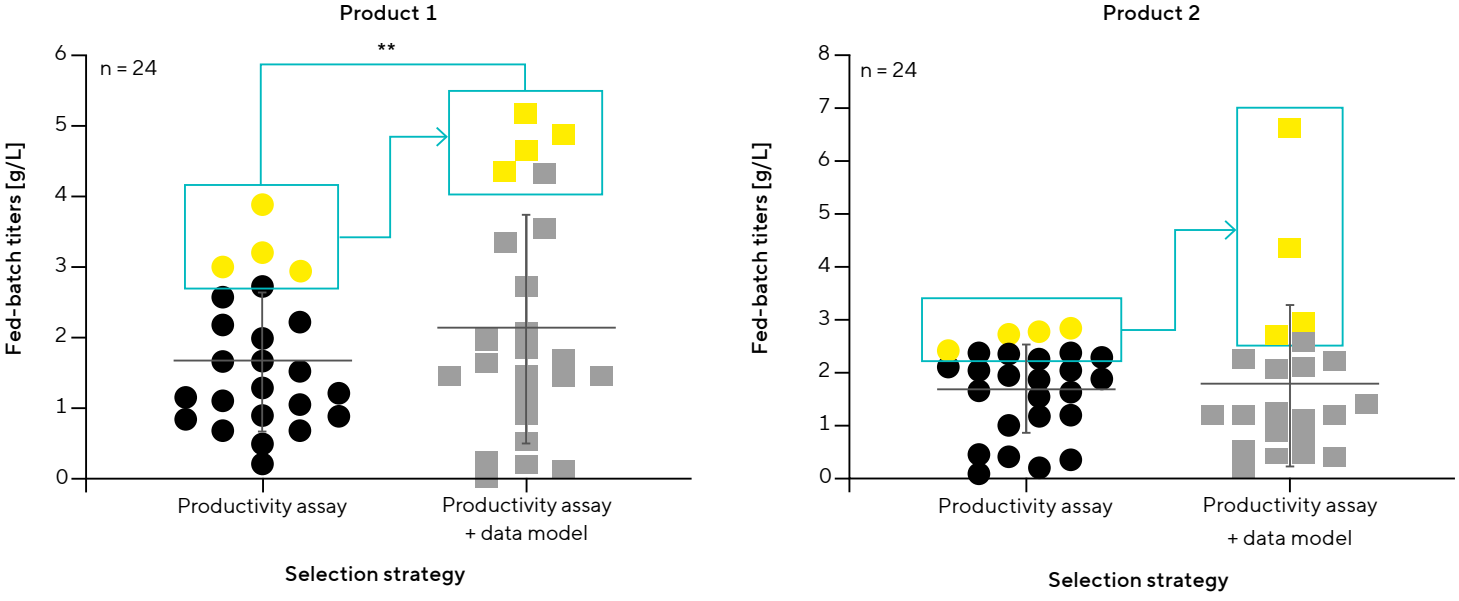
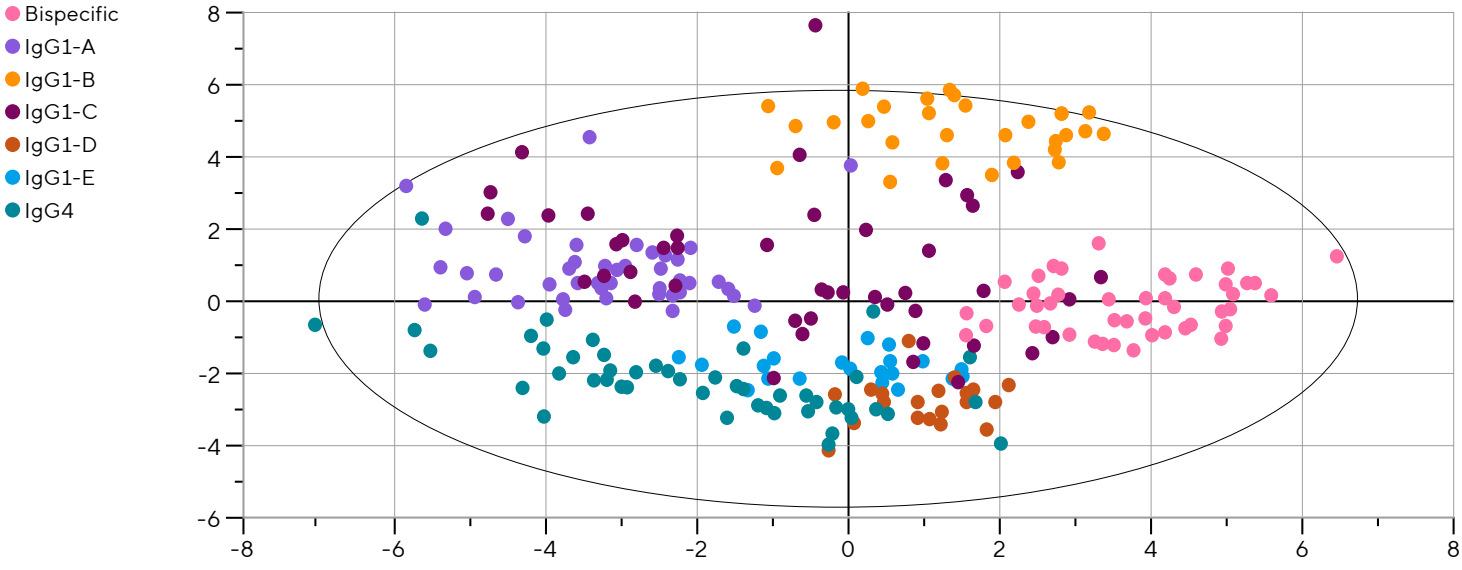


Figure 3 shows this strategy in action for two molecules. For product 1, the average titer of the top four clones increased by approximately 40%, a substantial and statistically significant difference. For product 2, the model enabled us to capture very high-producing clones that would have otherwise been overlooked. These insights are especially important for non-classical antibodies, where it is challenging to find clones with sufficient titers and productivity.

Clone evaluation: Maximizing the odds

The next stage is the clone evaluation, which is performed in Ambr® 15 systems. For classical proteins, screening 24 clones in parallel under fed-batch conditions is sufficient to identify top performers. However, for complex molecules, the number can be increased to 48 clones. Multivariate data analysis (MVDA) reveals that clones expressing the same molecules exhibit variations in their growth, titer, and product quality profiles (Figure 4). By enhancing the number of clones, we increase the chances of finding the optimal clone for both expression and product quality. This reduces process and media optimization efforts during later development phases.

Figure 4: Clone variation for different molecules in the Ambr® 15



Note. Modeled using SIMCA® software for seven different products. Parameters evaluated included growth, titer, and product quality profiles.

A Toolbox Approach Tailored for Complexity

Innovation and expertise are critical to a robust cell line development platform. For more complex projects, flexibility also becomes essential. Sometimes, achieving target titers, specific post-translational modifications, or the right conformation of subunits can be challenging. In these cases, individual solutions are necessary to meet clients' expectations.

A toolbox approach ensures that cell line development can meet the needs of diverse projects. Below, we outline some of the tools used in our CHO Cell Line Development Services that enable us to offer tailored solutions for even the most demanding campaigns.

Expression screening with mini- and large-pools

One way to reduce risk during the development of more complex protein molecules is to introduce an additional pool phase before beginning single-cell cloning. This enables our clients to assess expression levels and critical quality attributes (CQAs)—including low aggregation and fragmentation levels, as well as the correct assembly of the molecule—early in the development process. The appropriate pool strategy (large, heterogeneous pools vs. more homogeneous mini-pools) is determined based on the project demands.

The large-pool approach is advantageous for evaluating different amino acid sequences in parallel and identifying the best candidate to advance. It saves significant time and resources by enabling data-driven decision-making before progressing to single-cell cloning with the top candidate. The mini-pool approach allows screening of up to 24 more homogeneous pools in parallel for performance and product quality. When a specific target product quality profile must be achieved, establishing and screening preliminary mini-pools helps ensure that the target profile is met prior to progressing to single-cell cloning.

Analytical tools are required to quantify titer and critical quality attributes (CQAs) when evaluating stable pools or clones. The following sections outline the approaches used at Sartorius.

Octet® system for product quantification

For molecules that bind to Protein A or L, we have standardized protocols to measure titer along the complete cell line development workflow. We can also establish customized quantification assays with the Octet® system when the molecule does not bind to Protein A or Protein L. Here, we use Streptavidin biosensors and conjugate them with a biotinylated ligand specific to the target protein. This enables direct quantification of the molecule of interest in the crude supernatant. Our method replaces the previous standard enzyme-linked immunosorbent assay (ELISA), which was time-consuming, inflexible, and error-prone.

LabChip™ GXII for CQAs

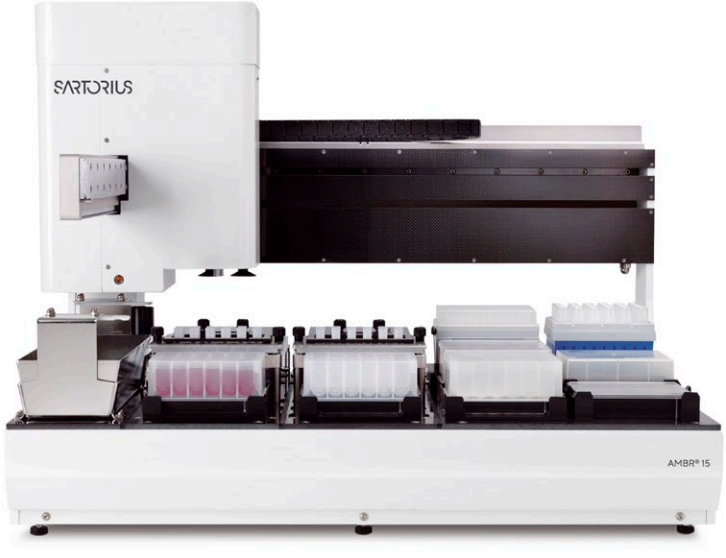
The LabChip™ GXII is employed for high-throughput screening of basic CQAs, including charge variance, molecular weight, and N-glycan, as well as ensuring the correct formation of the molecule. As early as six weeks after transfection, product quality analytics can be implemented at the small-scale fed-batch level, allowing rapid decision-making at the clone level.

Advanced analytical methods

More advanced approaches for in-depth protein characterization can be performed using high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS), surface plasmon resonance (SPR), and cell-based activity assays to determine a range of physicochemical and functional attributes.

Process and media optimization

If further improvements to titers or CQAs are required, we can fine-tune process parameters and media formulations using design of experiments (DoE) approaches in Ambr® 15 and Ambr® 250 systems. Using Sartorius MODDE® software, an optimal set of conditions is defined and evaluated for its impact on titer and CQAs. We can perform multiple rounds of DoE until the optimal CQA profile and best possible titer are achieved. A panel of more than 50 custom media formulations can also be screened to select the most promising formulation, depending on the optimization needs. In addition, we can choose either fed-batch or perfusion mode. To avoid increasing timelines, these optimizations can be carried out in parallel with the stability studies.

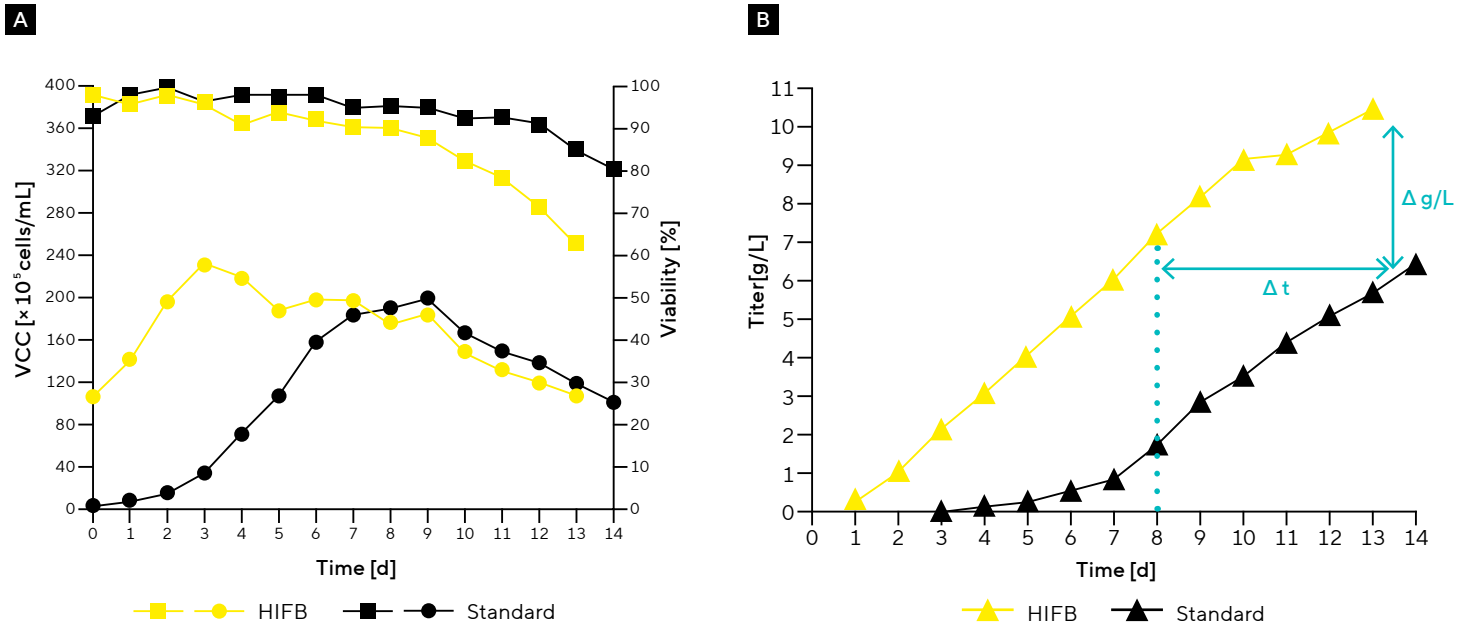


Process intensification:
Enabling higher productivity and flexibility

Process intensification has become an essential strategy for biopharmaceutical developers seeking to improve efficiency and reduce costs. This is particularly important in the context of complex proteins, where titers are typically lower, and molecules are often unstable in standard fed-batch processes. To meet these evolving needs, we have developed several process intensification tools that can be integrated into our cell line development platform.

By starting cultures at higher seeding cell densities (usually 30-fold higher than standard fed-batch), we can achieve faster biomass accumulation and higher peak titers without extending process duration (Figure 5). This either accelerates protein production or saves costs by shortening the production duration or increasing the yield per batch.

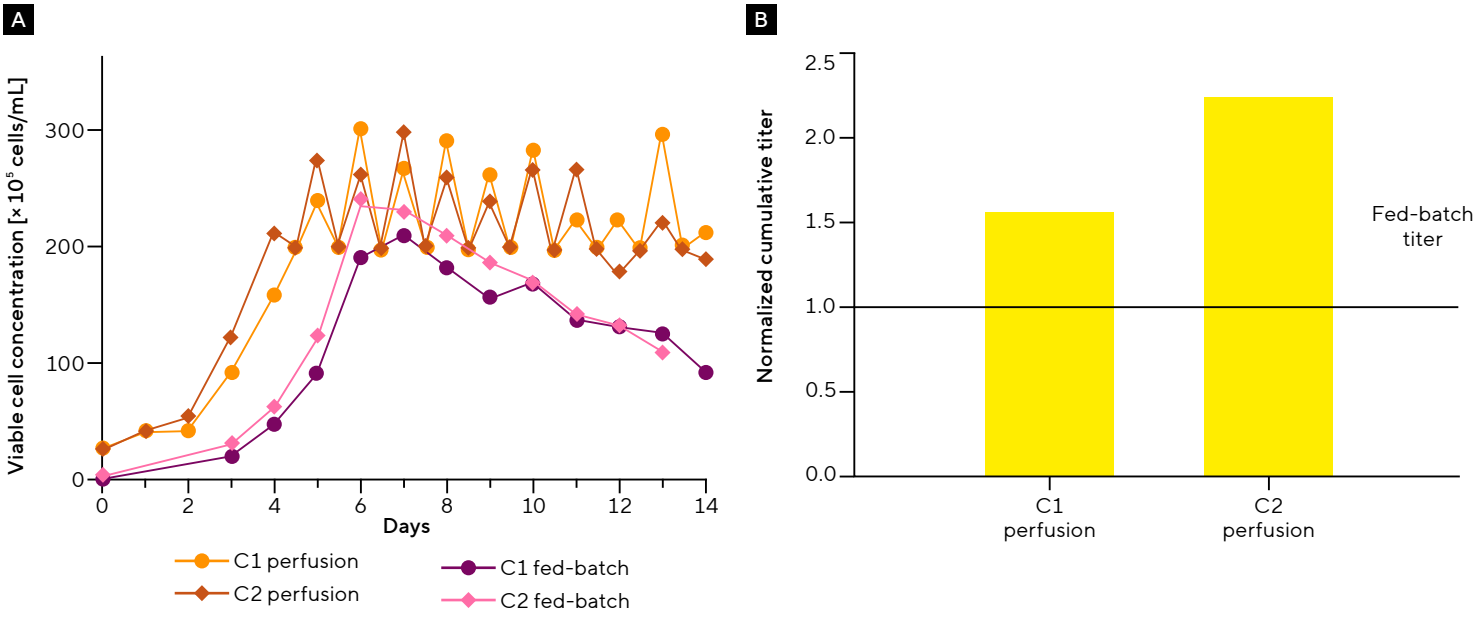
Figure 5: Comparison of **(A)** viable cell concentration and cell viability and **(B)** titer for HIFB and standard fed-batch cultures



Perfusion mimic workflows in Ambr® 15

We developed a perfusion-mimic process in the Ambr® 15 to allow us to evaluate clones for perfusion or continuous bioprocessing. The process is semi-continuous, with daily cell bleeds to maintain a constant cell density, followed by centrifugation and media exchange to remove both spent media and the product. This process can significantly increase the protein yield for products that are sensitive to degradation (by, for example, cellular proteases) or cytotoxic molecules.
Figure 6 shows two clones with increased titer following transition from a fed-batch to a perfusion mimic process in Ambr® 15. A true continuous perfusion process can also be performed in the Ambr® 250, supporting further optimization of clones selected from the Ambr® 15 screening process and ensuring robust scalability.

Figure 6: Comparison of two clones (C1 and C2) in fed-batch and perfusion mode. Viable cell concentration **(A)** and normalized cumulative titer **(B)** for two clones transitioned from fed-batch to perfusion mode. Clones were identified in fed-batch mode and then tested for suitability in a perfusion process.



Through both of these process intensification approaches, developers can achieve:
- Higher titers within the same process timeline
- Shorter process duration to reach target titers
- Improved product consistency and quality
- Reduced cost of goods (COGs) through optimized media and bioreactor utilization

Process intensification is especially valuable when scaling the production of complex proteins, helping to bridge the gap between development and commercial readiness while maintaining flexibility to adapt to molecule-specific needs.

Customer Case Study – Exceeding Expectations for a Difficult-to-Express Protein

Project brief

A client engaged Sartorius to develop a new CHO cell line for a difficult-to-express therapeutic protein. Due to tight project timelines, rapid progress was required.

Approach

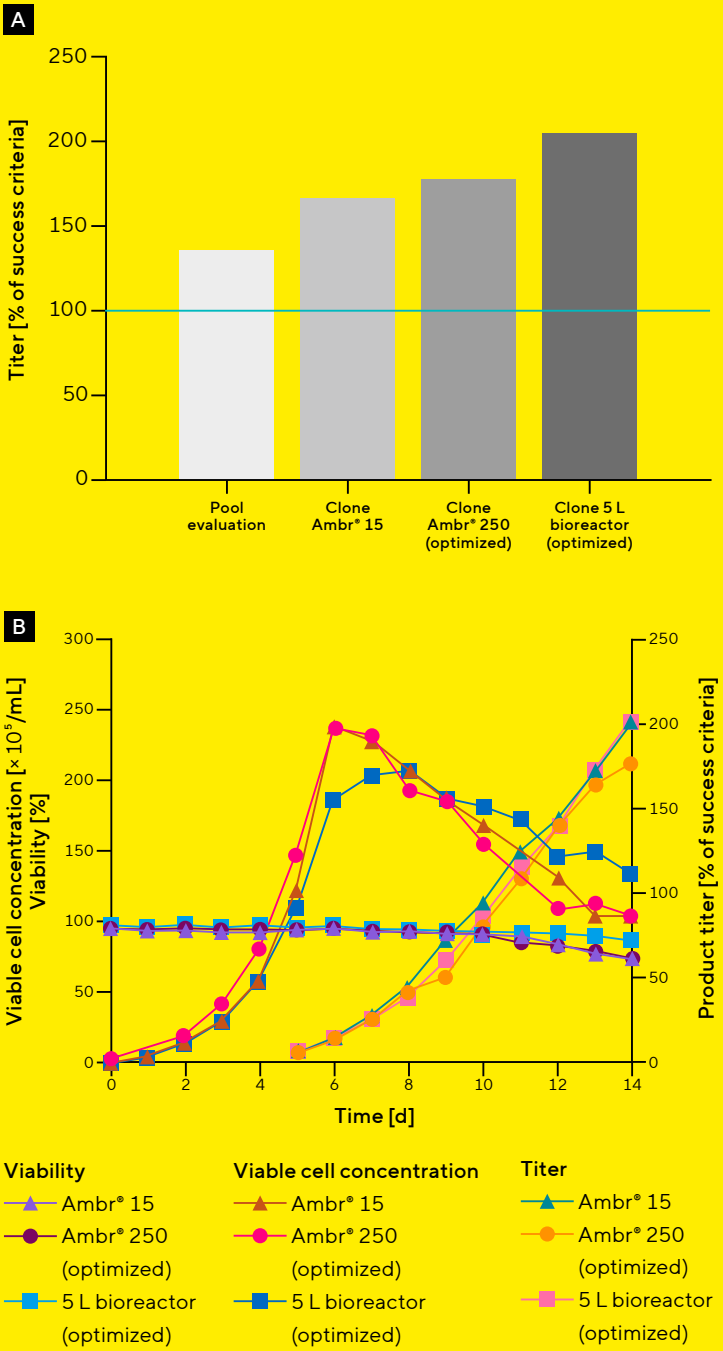
Given the non-standard nature of the target protein, we expected some challenges related to titer and product quality. As such, we implemented an additional pool phase to support pre-selection before single-cell cloning. In this case, we used our mini-pool approach, as the goal was to establish homogeneous pools with high titer and the desired product quality profile. Further adjustments to our platform process included varying seeding densities during mini-pool generation. This phase was extended by performing a small-scale fed-batch experiment to assess the behavior of a wider selection of pool variants under fed-batch conditions.

Following single-cell cloning and evaluation using the CellCelector™, including our productivity bead assay and AI | ML predictive model, we performed a DoE in the Ambr® 250. This process optimization allowed us to identify optimal bioprocess parameters before scaling up to a 5 L bioreactor. The top clones were also evaluated in an intensified process, using the Ambr® 15 perfusion mimic (Figure 6). This confirmed the suitability of those clones in the perfusion process.

Results

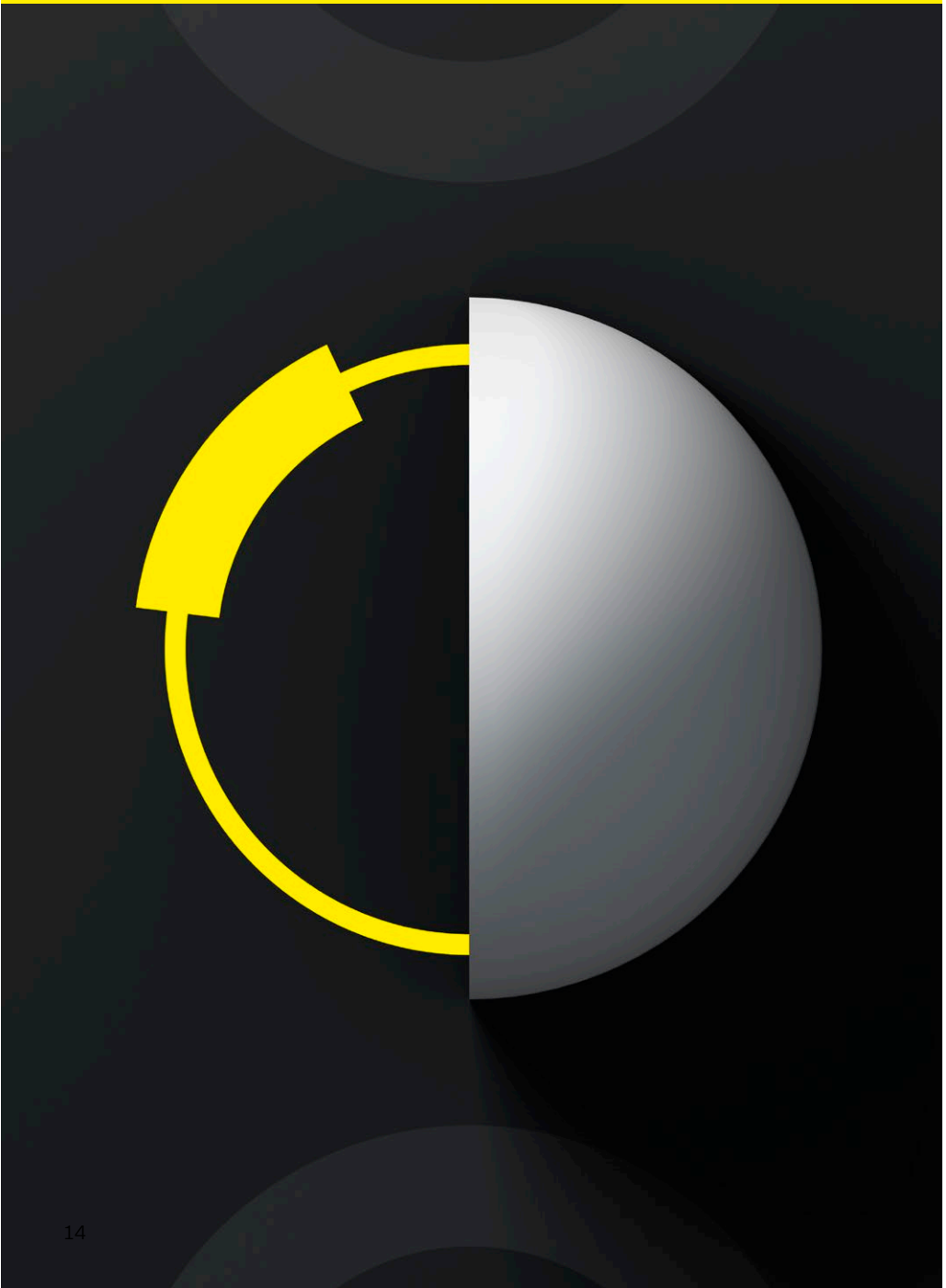
At the pool evaluation stage, titers already exceeded customer expectations (Figure 7A). Optimized parameters were identified during the DoE in the Ambr® 250 and led to further titer increases (~ 175%). The optimized settings were successfully scaled up to a 5 L bioreactor, resulting in a 200% increase in titer relative to the client's target. Process transfer to the client's contract development and manufacturing organization (CDMO) confirmed scalability up to 50 L. Viable cell concentration and viability showed robust scalability across the systems (Figure 7B).

Figure 7: Titer increase during cell line development **(A)** and comparison of viable cell concentration, viability, and titers between scales **(B)**.



Conclusion

Our CHO Cell Line Development Platform empowers biopharma developers to tackle the most complex protein formats with confidence. Across our programs, 29 non-standard protein entities developed using this platform have progressed into clinical phases, demonstrating its impact in real-world pipelines. Whether the challenge is expression, stability, or manufacturability, the platform delivers tailored solutions, predictive tools, and scalable performance—from DNA to RCB, and beyond.



Author Bio

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Yash D. Patel is a Product Manager for Cell Line Development at Sartorius, having joined the company in August 2021. He holds a PhD in chemical and biological engineering from the University of Sheffield and brings 15 years of experience in CHO cell line development and engineering.

Dr. Patel has worked with CDMOs such as Lonza and collaborated with AstraZeneca to patent novel cell line development technology and has authored numerous publications within the field. He is currently responsible for all product management activities related to CHO Cell Line Development Services at Sartorius, focusing on innovation, portfolio expansion, and supporting both research and product development teams.

Melanie Mann
PhD, Manager of Cell Line Development Operations, Sartorius

Melanie Mann is the Manager of Cell Line Development Operations at Sartorius, where she brings nearly a decade of experience in CHO cell line development for both biosimilars and novel biologics. She holds a PhD in Virology and studied molecular and cell biology at Erlangen University.

With a strong background in molecular and cell biology, bioprocess optimization, and technology transfer, she leads cross-functional teams to drive client projects from vector design through to robust research cell bank delivery. Melanie ensures scientific excellence at every stage, delivering fully characterized, scalable cell lines ready for clinical manufacturing.

Christiane Hartmann
PhD, Scientist – Cell Line Development Operations, Sartorius

With over five years of hands-on experience in CHO Cell Line Development, Christiane Hartmann supports Sartorius customers through every stage of their cell line development journey – from early feasibility to IND readiness.
She specializes in developing tailored solutions for non-standard and complex molecules, helping drug developers overcome challenges such as poor expression, low titers, and inconsistent product quality.

Christiane plays a key role in translating complex scientific workflows into actionable insights for our customers – whether through technical consultations, in-depth white papers, or practical webinars. Her deep technical understanding ensures each molecule gets the strategy and attention it needs to move forward confidently.

Katy McLaughlin
PhD, Marketing Communications Professional, Creative Operations, Sartorius

Katy is part of the Marketing Communications team at Sartorius, where she supports the creation of a variety of written pieces, from published articles to web content.

Before joining Sartorius in 2021, Katy was employed as a Post-Doctoral Research Associate at the University of Edinburgh, where she also completed her doctoral studies. Here, she carried out research in genetics and cellular biology and began taking on writing projects, eventually entering into a career as a freelance writer for various biotech companies and agencies.

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