Simplifying Progress

Sartorius 4Cell® CHO Platform CLD Service: Innovation Accelerating DNA to MCB to Generate High-Titer and High-Quality Cell Lines

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Introduction

Cell line development (CLD) is a decisive step in the production process of biopharmaceutical therapeutics like monoclonal antibodies (mAbs), setting the path for the future success of a molecule. While it has always been critical to achieve fast and efficient identification and development of a high-producing, stable cell line, time-to-clinic became even more relevant during the COVID-19 pandemic. Additionally, many platforms don't offer a reliable and efficient method of identifying high producers early in the CLD timeline.

In this study, our goal was to implement a high-throughput technology for the single cell cloning step that supports improved clone outgrowth, early prediction of high producers, and fast clone expansion. While developing the 4Cell® CHO CLD Platform, we established an automated single cell cloning method using the CellCelector® platform developed by Automated Lab Solutions; now part of Sartorius. We also implemented a fluorescence-based productivity assay. We combined this with a 96-well scale-down fed-batch approach to evaluate main process characteristics and clone productivity. With this CLD process setup, we were able to improve cell outgrowth, demonstrate early recognition of high-producer clones achieving up to 10 g/L, and reduce the CLD timeline to 9 weeks.

4Cell® CLD Workflow

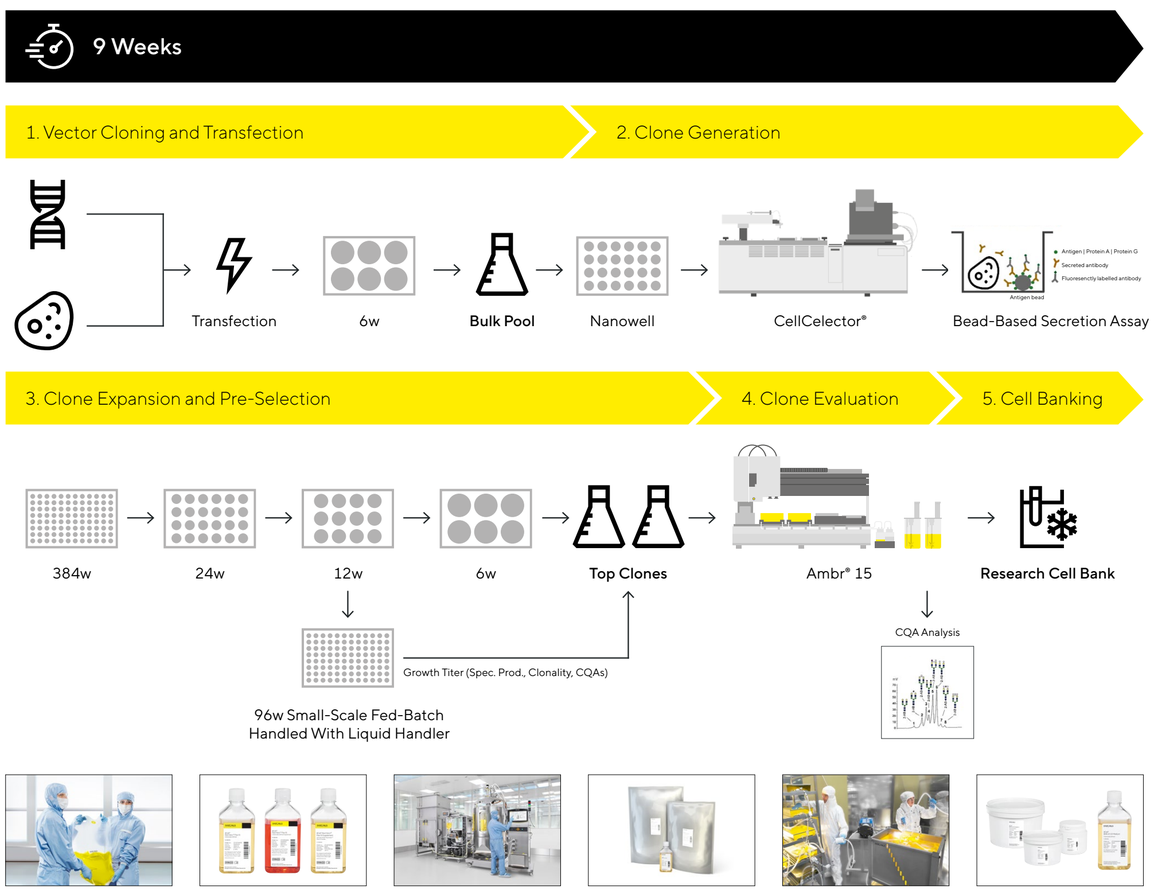

Figure 1: *Overview of the 4Cell® CHO Cell Line Development Process From DNA to Research Cell Bank in Nine Weeks, Including the CellCelector® Platform and the 96-Well Small-Scale Fed-Natch. 4Cell® SmartCHO Media Provided Robust Growth and High Productivity*

Within the 4Cell® CLD workflow (Figure 1), we transfected our CHO-DG44 host cell line with our proprietary expression vector carrying the gene of interest of the desired product. Single clones from the bulk pool were laid out in 24-well nanowell plates and visually analyzed with the CellCelector® platform. The included software collected various cell characteristics and images to prove monoclonality – together with fluorescence parameters from the Protein-A bead productivity assay, these were used to identify high-producing clones. The selected clones were transferred to 384-well plates and were further expanded to 12-well plates.

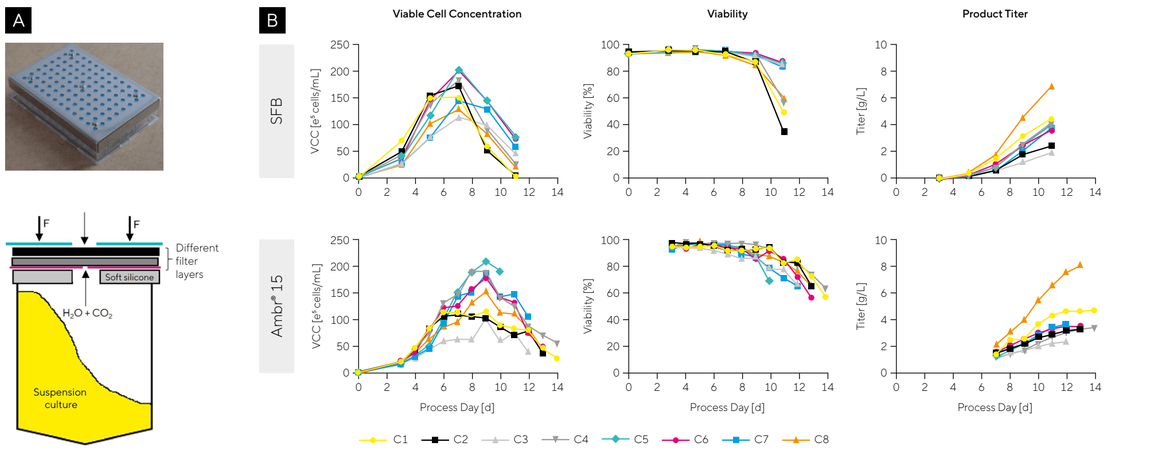

Figure 2: *(A) Schematic Description of the Duetz Plate Lid System Covering the 96-Well Deep-Well Plates for a Small-Scale Fed-Batch. The Filter Layers Prevent Liquid Evaporation While Ensuring Oxygen Transfer to the Cell. (B) Eight Clones Were Used to Evaluate the Setup of the Small-Scale Fed-Batch Approach and Revealed Comparable Growth, Viability, and Product Titers for Each Tested Clone in the Ambr® 15 Bioreactor*

Using a liquid handler, we inoculated 96 wells of a deep-well plate with some of the cells to run a fed-batch approach. For these 300 µL cultures, we used the Duetz plate cover system (Figure 2A). The 96-well fed-batches served as a scale-down model for the Ambr® 15 bioreactor (Figure 2B) and were used to further fine-tune the preselection of clones derived from the CellCelector® platform.
By considering growth and productivity data from the small-scale fed-batches, we further narrowed down our selection of clones. We evaluated these top clones for their main process characteristics using the Ambr® 15 bioreactor. Combined with critical quality attributes (CQA), this dataset can be used to define the top four clones for preparing the research cell bank (RCB).

Results

Improved outgrowth in 384 well using the CellCelector® platform and fluorescence microscopy, leading to higher survival of high producers compared to FACS

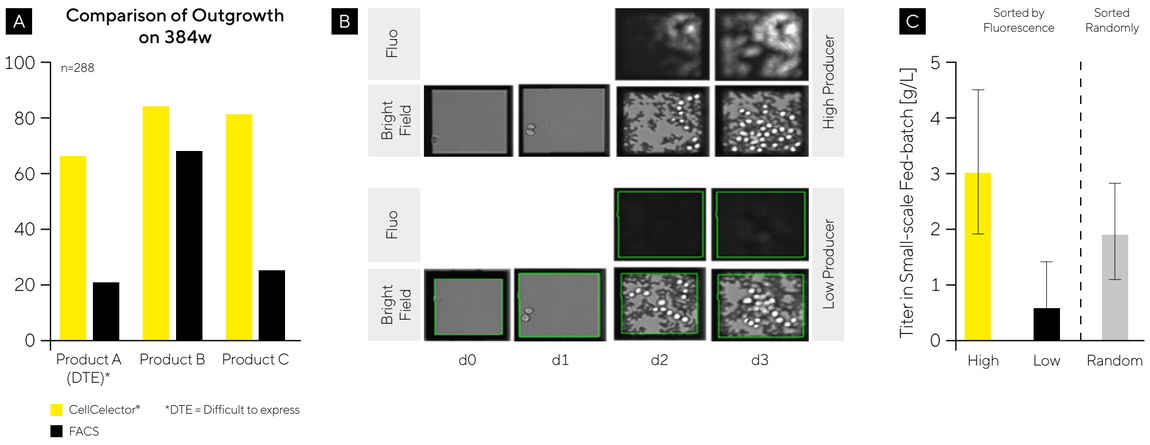

Figure 3: *(A) Evaluation of High and Low Producers in 96-Well Small-Scale Fed-Batch Compared to a Random Clone Selection Without Fluorescene Assay. (B) Comparison of Outgrown Clones Generated by the CellCelector® Platform and Facs Device on 384-Well Stage Tested With Three Products. (C) Bright-Field and Fluorescence Images of High and Low Producers at the Nanowell Stage*

Combined with the Protein A-based fluorescence assay, the CellCelector® platform enables detection of high-producing clones as early as three days after single cell cloning in a fed-batch environment (Figure 3B and C).
Using the generated images and analysis software, the technology provides a robust method to ensure monoclonality of the selected clones at 384-well stage. Due to the very mild conditions, clones selected using the CellCelector® platform showed better outgrowth in 384-well plates (Figure 3A).

96-well small-scale fed-batch as a second tool to find high producers and to predict molecule performance

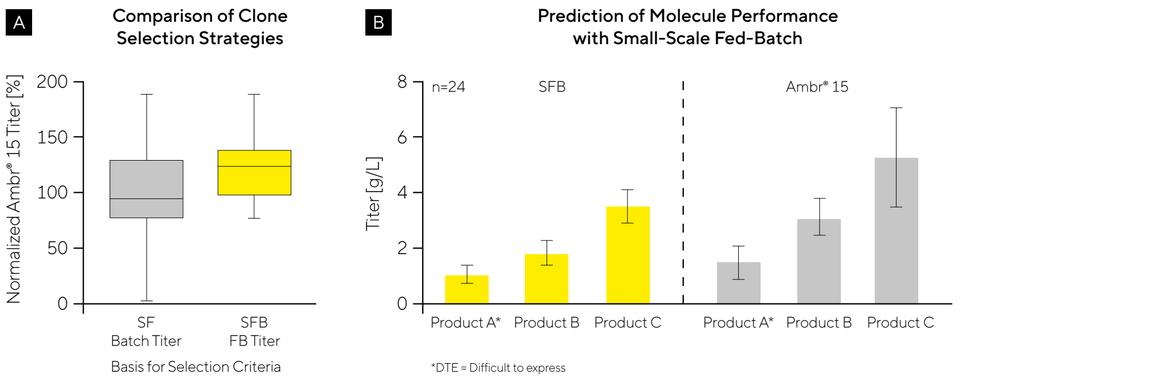

Figure 4: *(A) Comparison of Clone Selection Strategies for Ambr® 15 Bioreactor Clone Evaluation. (B) Predicition of Molecule Performance in the Ambr® 15 Bioreactor Using Small-Scale Fed-Batches*

Performing small-scale fed-batch to select top clones for further evaluation in the Ambr® 15 bioreactor increases the probability of finding a high-titer clone compared to selection using batch titers on shake flask level, and enables you to deselect low- and non-producers (Figure 4A). As demonstrated with three products, data obtained with small-scale fed-batches can provide early prediction of the molecule's later performance in the Ambr ® 15 bioreactor (Figure 4B).

Demonstrated consistent performance of up to 10 g/L using 4Cell® CHO CLD platform while solving challenges of difficult-to-express molecules

Figure 5: *Evaluation of Lead Clones Expressing the Three Test Products in a 14-Day Ambr® 15 Run In Fed-Batch Mode. Data Is Shown for Viable Cell Concentration, Viability, and Product Titer All Achieved 4Cell® SmartCHO Media*

Additional Services

- Gene Knockout Service – FUT8 KO Service Implemented for Increased ADCC
- High-Inoculation Fed-Batch | Save Time or Increase Titer

Conclusion

Speed: The CellCelector® platform significantly reduces the time needed to generate high-producing single cell clones, enabling DNA to RCB in nine weeks.

Better value: Shorter timelines and less need for consumables help reduce cost of goods.

Reliability: Combining the CellCelector® platform with 96-well small-scale fed-batch provides a reliable approach for identifying high producers.

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