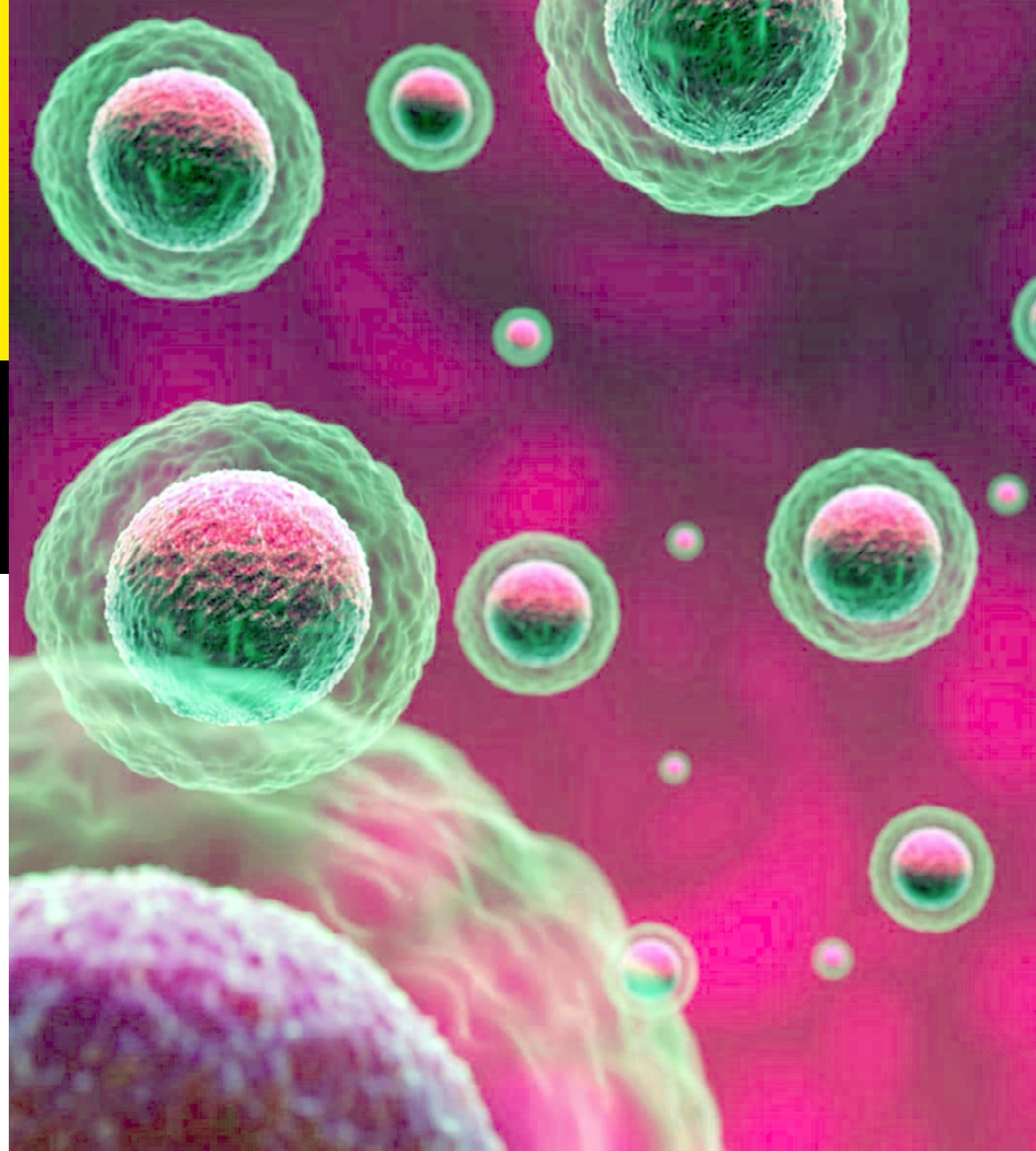
SARTORIUS

Simplifying Progress

Maximizing Efficiency in Cell Line Development

Early Productivity Screening to Guide Decision Making.

Get Started



Introduction

The CellCelector platform revolutionizes cell line development (CLD) by integrating single-cell isolation, automated imaging, and early productivity assessment into a unified, high-throughput workflow. By utilizing advanced nanowell technology, this system significantly outperforms traditional limiting dilution and FACS methods by delivering superior monoclonality verification, enhanced clone outgrowth, and earlier access to critical secretion data. These combined capabilities allow laboratories to maximize efficiency, reduce resource consumption, and ensure high-quality clonal selection from the earliest stages of development.

We will also explore how other Sartorius instruments enable scientists to gain insights into product quality early in cell line development and avoid costly setbacks further downstream. From the CellCelector and the Ambr® 15 platforms for single-cell cloning and culture optimization, to the Octet® BLI platform for clone characterization, Sartorius simplifies the CLD process and helps users identify the winning clones with confidence.

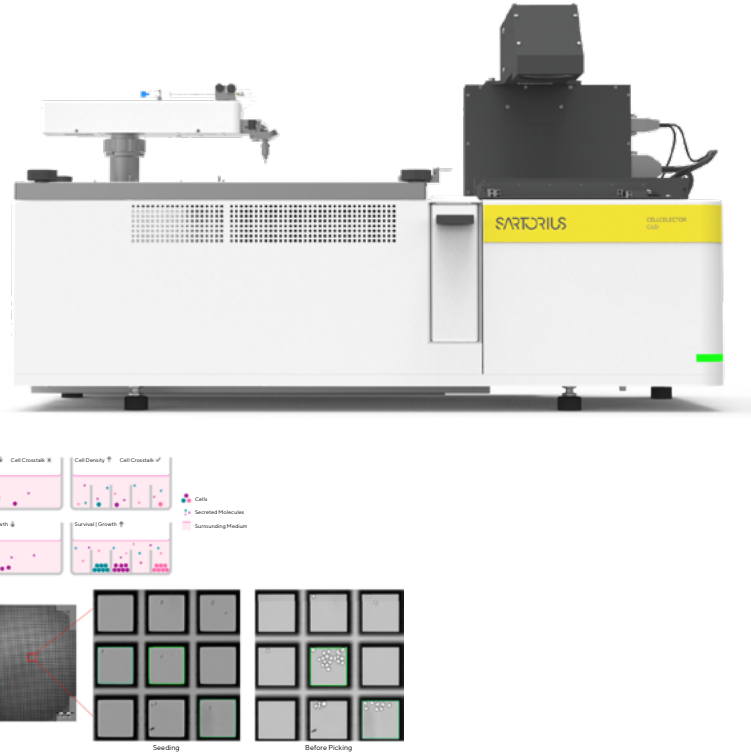
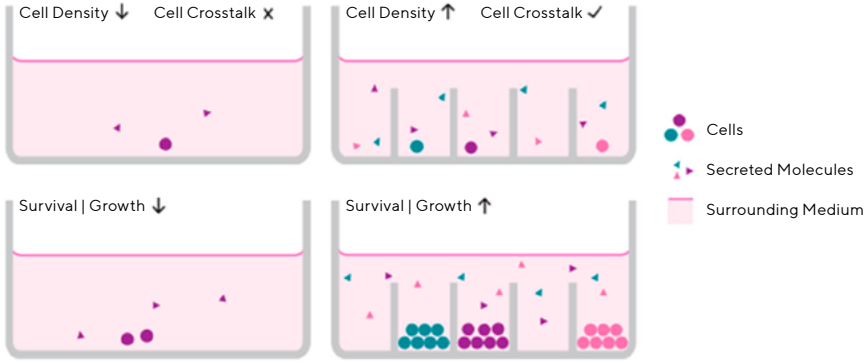


Figure 1: CellCelector Nanowells Facilitate Decreased Resource Requirements During CLD

Click to Expand

A



B

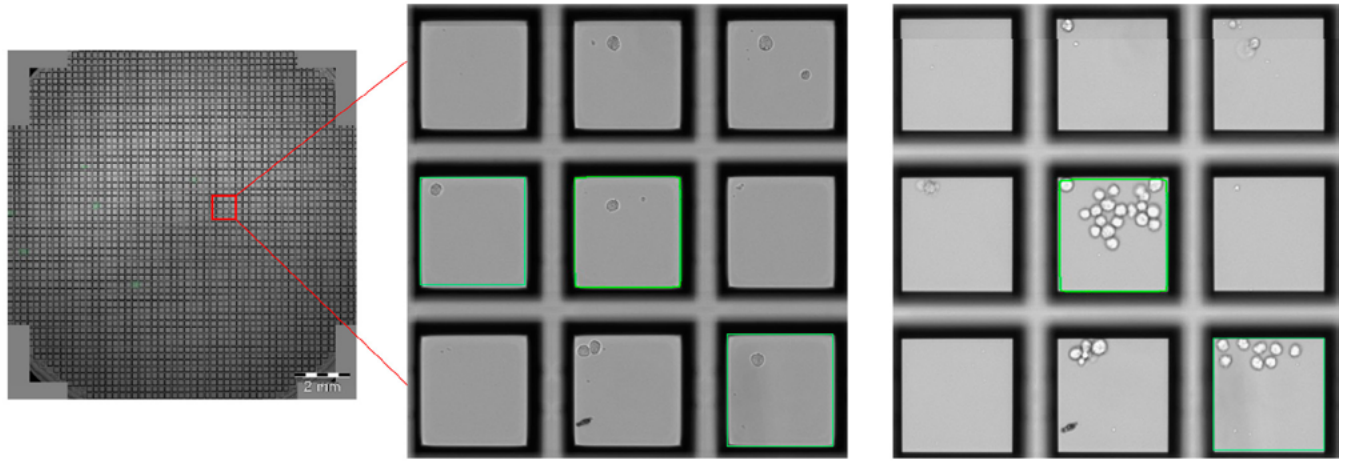


Figure 1: *CellCelector Nanowells Facilitate Decreased Resource Requirements During CLD.*

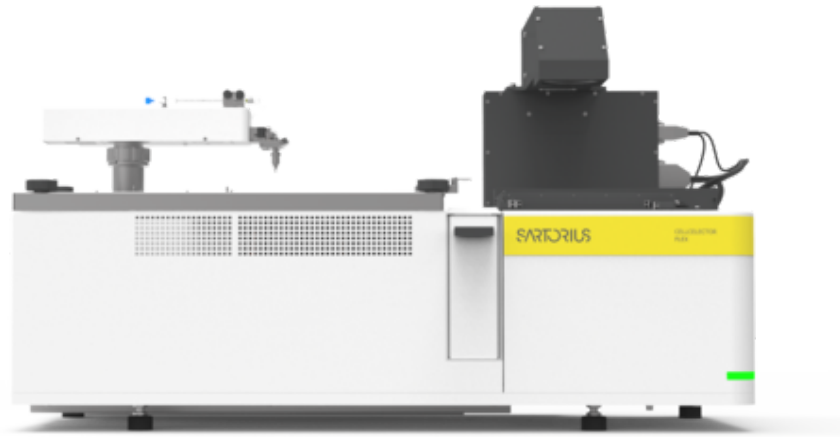
(A) Schematic detailing the benefits of the CellCelector nanowells over standard well formats. **(B)** Micrographs illustrating the CellCelector nanowell plate structure with up to 4000 individual nanowells per well of a 24-well plate format array.

Chapters

Introduction | Methods | Results | Conclusion

Application Note: Facilitating Rapid, Accurate Cell Line Development with the CellCelector Platform

Daryl Cole, PhD – Scientist – BioA Applications – Sartorius
Darius Wilson, PhD – Manager – Product Management – Sartorius
Nicola Bevan – Manager – BioA Applications – Sartorius



Chapters

Introduction | Methods | Results | Conclusion

Introduction (Part 1)

Generating biologics often requires the development of cell lines capable of producing the molecule of interest. The manufacture of a cell line that can be successfully cultured, is robust, and produces the biotherapeutic required at a high enough yield are all key components to a successful cell line development (CLD) workflow. Many methods to produce these cells of interest in high numbers, are time-consuming, resource intensive and prone to human error or operator inconsistencies. Automation is becoming more prevalent in CLD due to the ability to increase both throughput and accuracy.

CLD techniques, such as limiting dilution (LD), traditionally require large quantities of reagents and consumables to achieve single cell seeding as required to create new cell lines. The CellCelector mitigates this issue by monitoring clones and ensuring monoclonality from the outset. The key to guaranteeing monoclonality lies in the utility of nanowell technology. CellCelector nanowells are microscopic wells contained within a standard well of a culture plate, for example, one well of a 24-well plate can contain up to 4,000 individual nanowells (Figure 1). This improves efficiency during CLD compared to traditional methods for single cell cloning, by ensuring enough single cells are present for developing clones with the use of only one plate of a 24-well nanowell system. This single plate has the potential to produce around 7,500 viable clones with proven monoclonality, while conventional LD might provide approximately 400 viable clones that still require monoclonality testing using as many as 90x96-well plates. Additionally, the cells cultured in the nanowell system benefit from shared signalling molecules and other factors, resulting in increased survival and growth as they are physically separated, but not isolated from their neighboring clones. The advantages to the nanowell approach are numerous, such as reductions in growth media requirements, space required for plates, time to culture and increases in cell health, expansion potential and titer. Other approaches to CLD involve different methods for single cell selection for monoclonal culture of clones of interest.

Introduction (Part 2)

Chapters

Introduction (Part 2)

Techniques such as Fluorescence Assisted Cell Sorting (FACS) allow the automated selection and seeding of clones as single cells for monoclonal culture, but this method is slow and harsh on cells, often resulting in lower clone viability and growth rate. The CellCelector can mitigate these issues because the picking and seeding of cells with the platform is very gentle, maintaining the integrity of clones for very high levels of outgrowth, viability and yield.

Determining clone product yield is usually performed at later stages in the CLD workflow, after small scale expansion phases are undertaken on promising clones. The problem with this practice is the potential waste in expansion of unsuccessful clones for characterization. The CellCelector introduces a new method for characterizing clonal yield potential at very early stages of CLD using a beadbased secretion assay in combination with nanowell technology. This technique mitigates the waste associated with conventional methods and allows the best clones to be selected and taken to expansion stage, saving time and resources.

Conventional methods for cell line development, such as FACS single cell selection, manual clone selection and traditional LD cloning, can:

- Result in low clone viability and outgrowth, while requiring multiple platforms to optimize culture conditions
- Require a number of small-scale clone expansions to quantify production yield prior to large scale expansion, increasing costs and time to produce viable clones
- Necessitate the use of multiple time and resource intensive techniques to monitor different clone product yields, viability and outgrowth during the CLD process
- Be inefficient, conventional LD requires large numbers of cells and consumables to ensure a viable percentage of single cells are seeded, while monoclonality still needs to be proven via imaging

This application note outlines the utility and value of the CellCelector at key stages of the CLD workflow.

Introduction (Part 1)

Chapters

Introduction | Methods | Results | Conclusion

Methods

The following methods outline the use of the CellCelector in key CLD processes, from single cell cloning and monoclonality monitoring, to characterization of final cellular products. Figure 2 summarizes the CLD workflow using the CellCelector Nanowell technology.

Cell Culture and Maintenance	Click to Expand
Cell Line Culture	+
Fed Batch Outgrowth Experiments	+
Titration and Yield Analysis	+
CellCelector	+
Bead-based Secretion Assay	+
Fluorescence Assisted Cell Sorting (FACS)	+

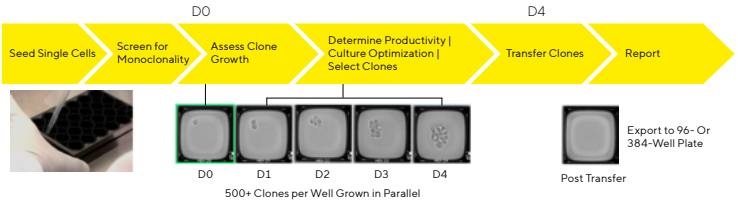


Figure 2: CellCelector Nanowell CLD Workflow.

+ **Click to Expand**

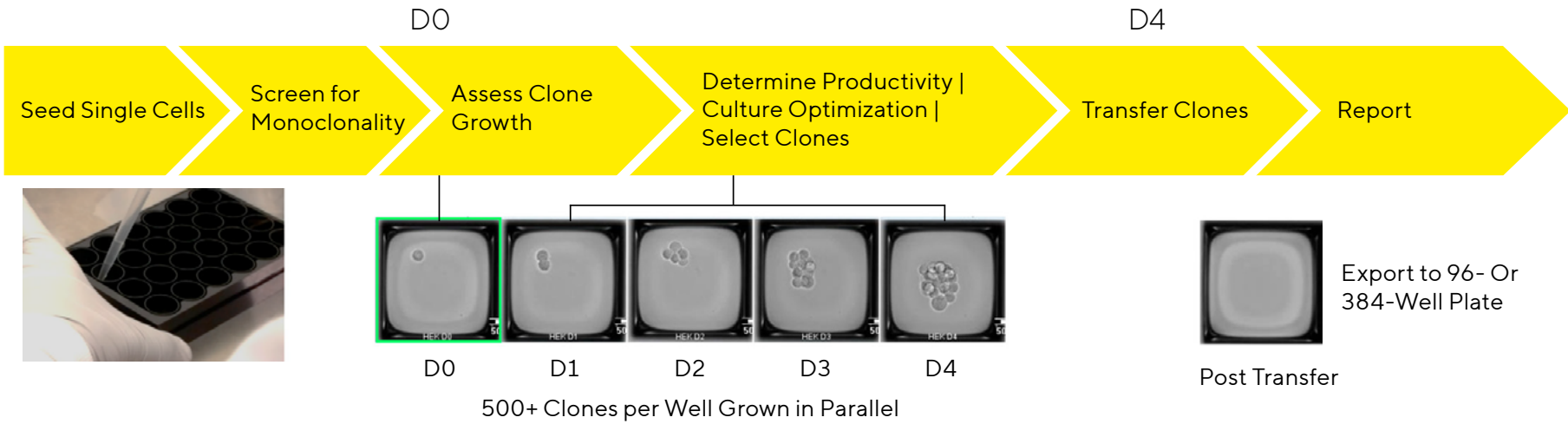


Figure 2: *CellCelector Nanowell CLD Workflow.*

Schematic depicting the CellCelector CLD workflow, highlighting key points in the process and images taken by the CellCelector for monoclonality screening and monitoring clone growth.

Chapters

Cell Line Culture

Chinese Hamster Ovary (CHO) DG44 cells were grown in T75 flasks at 2x105/mL in 10 mL complete medium consisting of F-12K plus 10% FBS. Passaging was performed using Trypsin-EDTA by adding 3 mL to a T75 flask until the cell layer dispersed in up to 15 minutes. A subcultivation ratio of 1:4 was used for each passage. Seeding into anowells was performed manually by seeding CHO cells directly into each well of a nanowell plate at 2x103 cells/mL, 1 mL per well. Cells were prepared using a cell strainer to ensure all clumps or aggregates were removed from the single cell suspension prior to seeding.

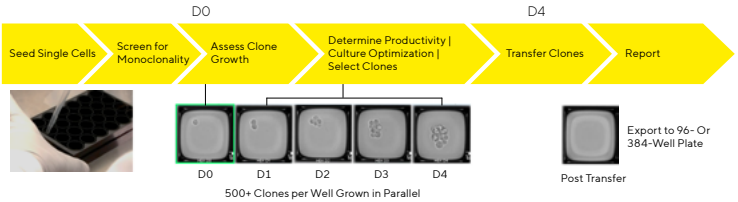


Figure 2: CellCelector Nanowell CLD Workflow.

Click to Expand

Chapters

Fed Batch Outgrowth Experiments

To quantify cell outgrowth, six different CHO cell clones were selected, and single cell seeded using conventional FACS or CellCelector picking methods and the outgrowth of these cells was measured using the CellCelector microscope and analysis software. CHO cells that were successfully picked using the CellCelector were transferred to 96-well plates in 300 µL complete medium and incubated at 37°C using the Duetz culture plates and cell shaker system. Supernatants were sampled for product titration analysis.

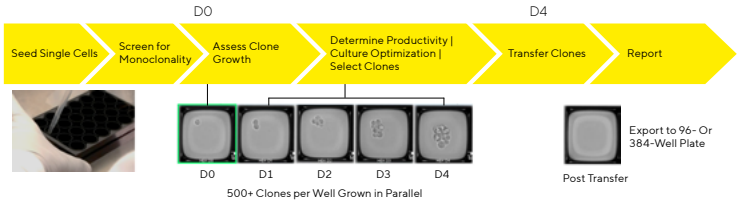


Figure 2: CellCelector Nanowell CLD Workflow.

Click to Expand

Chapters

Titration and Yield Analysis

Titration analysis was performed using the Octet® R8 system and the Protein A or G biosensors to determine yield of bioproduct. Characterization and monitoring of monoclonality.

Learn More

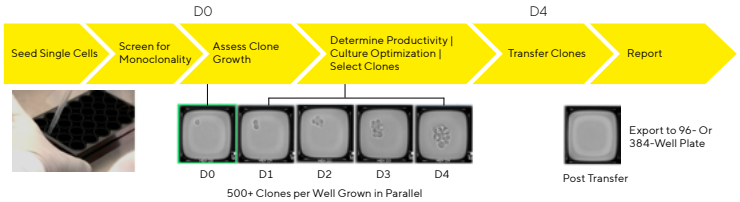


Figure 2: CellCelector Nanowell CLD Workflow.

Click to Expand

Chapters

CellCelector

The CellCelector was used to automatically analyze and pick cells from nanowell plates for single cell seeding after monoclonality analysis. Plates were very gently removed from the incubator and placed into the source plate stage above the inverted microscope. Cells were then analyzed for monoclonality and subsequently picked using the Single Cell Picking Module and seeded into 96- or 384-well plates for expansion.



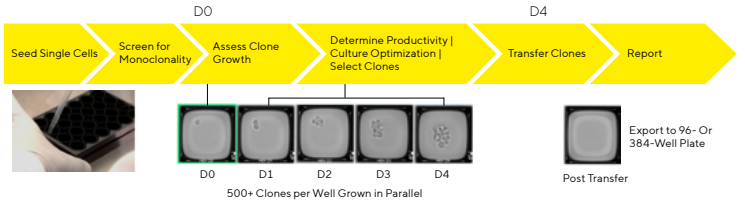


Figure 2: CellCelector Nanowell CLD Workflow.

Click to Expand

Chapters

Bead-based Secretion Assay

Specific antigen beads, e.g. Protein-A assay Spherotech Cat.-# PAP-40-5 with bead sizes of 4.0 – 4.9 µm, for the product of interest, were added to cells seeded in nanowells alongside a secondary, fluorescently conjugated antibody targeting the product.

When the product of interest is secreted from cells, it binds to the antigen bead. The secondary antibody then binds the product, resulting in a fluorescent signal. This signal provides an indicator of the production level of each clone tested, so high and low producing clones can be discerned, while non-producers can be discarded. For each well to be seeded, 500 µl of bead/antibody suspension mix was prepared.

Antigen or Protein-A / Protein-G coated beads were made up at a concentration of 250 µg/ml and mixed with Alexa Fluor 488 (or other fluorescence dye)-conjugated detection antibody made up at a concentration of 5 µg/ml. (Make sure to use F(Ab')2-fragment detection antibody in case of generic Protein-A/Protein-G coated beads to avoid direct binding of the detection antibodies' Fc-area to the detection beads.) The bead/antigen mix was carefully added dropwise to the nanowells, and the plates were incubated for 1 to 3 days until the fluorescence signal was clearly visible.

Learn More

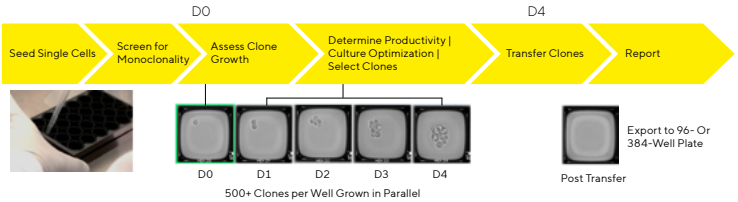


Figure 2: CellCelector Nanowell CLD Workflow.

Click to Expand

Fluorescence Assisted Cell Sorting (FACS)

The FACSAria Fusion, from BD, was used as a conventional method for single cell selection and seeding in comparison with the CellCelector.



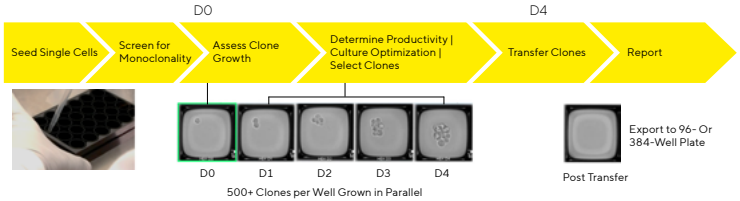


Figure 2: CellCelector Nanowell CLD Workflow.

Click to Expand

Results

CLD can be a long and arduous process that does not always yield high quality results, problems can arise with ensuring monoclonality, successful clone outgrowth and quantity of final product. The data presented here showcase the benefits of the CellCelector as a platform for enhanced CLD workflows, incorporating nanowell technology for single cell seeding, monoclonality monitoring and characterization of product yield.

Click to Expand

- Quantifying Clone Outgrowth for Process Optimization
- Analysis of Clone Products Using the CellCelector Nanowell-Based Secretion Assay
- Confirming Clone Selection Based on Yield Performance Prediction
- Characterizing a Range of Product Producing Clones Using the CellCelector

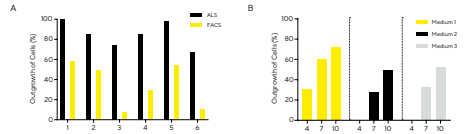


Figure 3: Clone Outgrowth Comparisons Exemplify the Use of CellCelector as a Solution for Cell Line Development.

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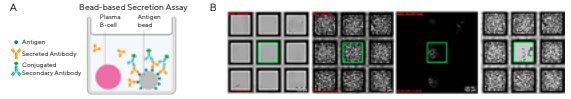


Figure 4: Clone Product Analysis Implementing a CellCelector Nanowell-Based Secretion Assay.

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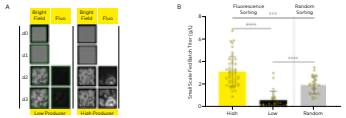


Figure 5: Confirmation of Performance Prediction and Clone Selection.

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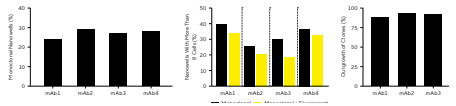


Figure 6: Confirmation of Outgrowth in Nanowells During CLD.

Click to Expand

Quantifying Clone Outgrowth for Process Optimization

Quantification of clone outgrowth is essential during the CLD workflow because it provides key metrics related to the users clones of interest, viability and proliferation potential. This is an important factor in choosing a successful clone because it highlights those clones that will grow sufficiently and ultimately produce higher yields. Determining this earlier, results in reduced resource costs associated with developing non-optimal clones.

The data in **Figure 3A** shows that cellular outgrowth using the CellCelector is consistently more than 40 – 50% higher than FACS picking for all the clones tested.

Figure 3B shows the outgrowth of cells using the CellCelector with a range of media formulations. Here we can see that over 4,7, and 10 days of culture, media formulation 1 provided the highest percentage outgrowth of cells, indicating that it is optimal for culture of this CHO cell line. The data in this figure exemplifies the capabilities of the CellCelector for cell picking and for optimization experiments on key culture conditions during CLD workflows.

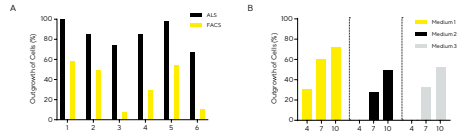


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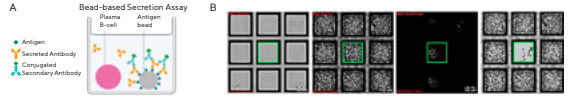


Figure 4: Clone Product Analysis Implementing a CellCelector Nanowell-Based Secretion Assay.

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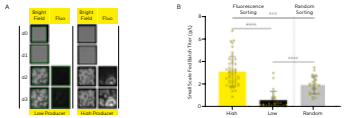


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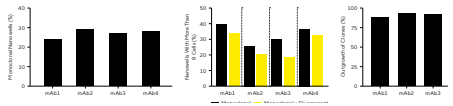


Figure 6: Confirmation of Outgrowth in Nanowells During CLD.

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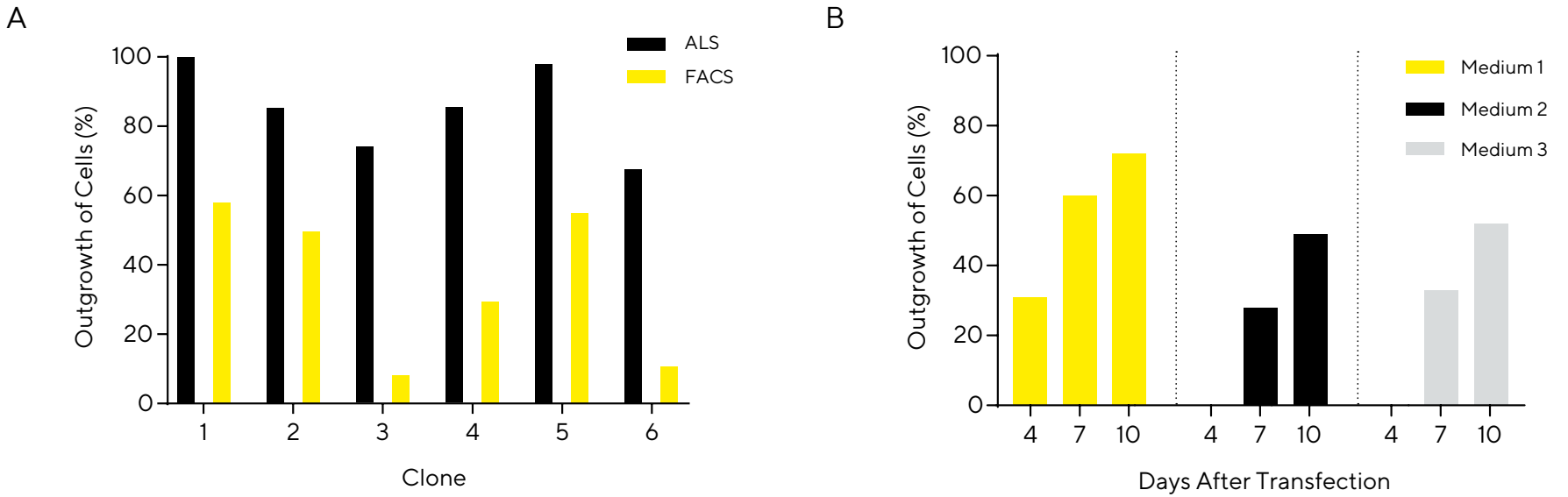


Figure 3: *Clone Outgrowth Comparisons Exemplify the Use of Cellcelector as a Solution for Cell Line Development.*

(A) Bar graph comparing the outgrowth after 35 days of a number of CHO DG44 clones selected using Fluorescence Assisted Cell Sorting (FACS) or the CellCelector.

(B) The CellCelector can be effectively used in media formulation optimization experiments by monitoring clone outgrowth to determine the optimum conditions for the user's cell lines.

Chapters

Analysis of Clone Products Using the CellCelector Nanowell-Based Secretion Assay

Fully characterizing your clones as early as possible during CLD is very important before moving to expensive and resource intensive expansion and upscaling. Conventional CLD methods usually employ small scale expansions of promising clones and quantify production yield to determine which clones to progress to full scale expansion and production. The CellCelector Nanowell-based secretion assay, in contrast, enables fast and simple characterization of clone outgrowth capability and potential future yield of product without the need for expansions at very early stages of the CLD workflow.

CHO cell clones were grown from single cells in nanowells for 3 days prior to collection for expansion. Using the beadbased secretion assay, the level of product produced by each clone was analyzed as early as day 3 in combination with cell outgrowth.

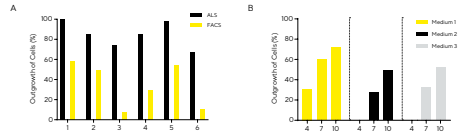


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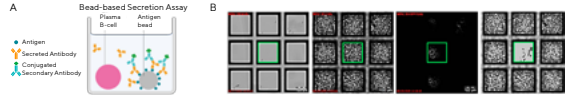


Figure 4: Clone Product Analysis Implementing a CellCelector Nanowell-Based Secretion Assay.

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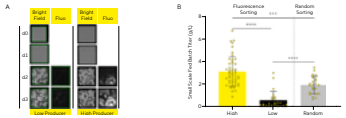


Figure 5: Confirmation of Performance Prediction and Clone Selection.

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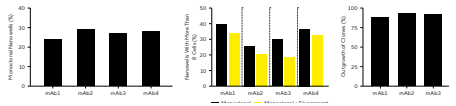


Figure 6: Confirmation of Outgrowth in Nanowells During CLD.

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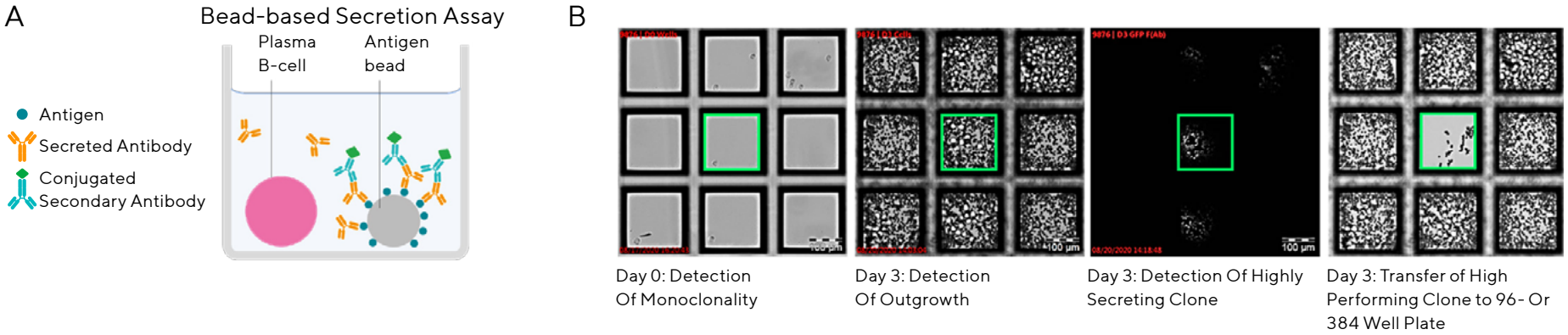


Figure 4: *Clone Product Analysis Implementing a CellCelector Nanowell-Based Secretion Assay.*

(A) Schematic of the Bead-based Secretion Assay.

(B) Micrograph images from the CellCelector showing the selection process for a high producing clone.

Confirming Clone Selection Based on Yield Performance Prediction

To do this, clones were selected based on their fluorescence levels in the nanowell secretion assays as either low producers (low fluorescence) or high producers (high fluorescence). Figure 5A shows the process of selecting a monoclonal nanowell and their growth over 3 days. At day 2,nthe antigen beads are added to culture and fluorescence levels are analyzed for 24 hours before cells are characterized as a low or high producer and picked for expansion. The data shows that both the low and high producers are monoclonal when initially seeded and that they expand into clones producing the antibody of interest. Figure 5B confirms the initial characterization shown in 5A, in small scale fed batch titer levels. As the box plot illustrates, cells classified as high producers in the secretion assay produce significantly higher titers of antibody at the expansion stage than low producers. High producers also present a significantly increased titer than standard random selection (30% higher titer, Figure 5B). Further, the data for both low and high producers is significantly different to the randomly selected control.

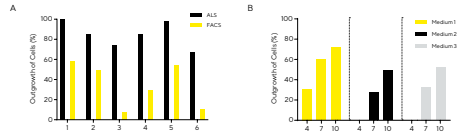


Figure 3: Clone Outgrowth Comparisons Exemplify the Use of CellCelector as a Solution for Cell Line Development.

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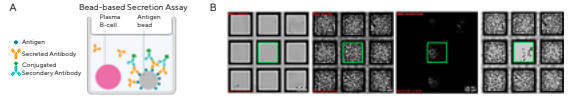


Figure 4: Clone Product Analysis Implementing a CellCelector Nanowell-Based Secretion Assay.

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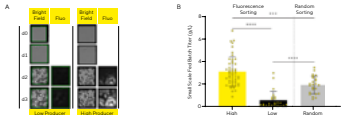


Figure 5: Confirmation of Performance Prediction and Clone Selection.

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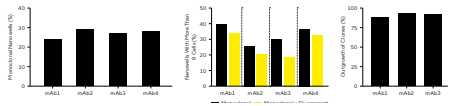


Figure 6: Confirmation of Outgrowth in Nanowells During CLD.

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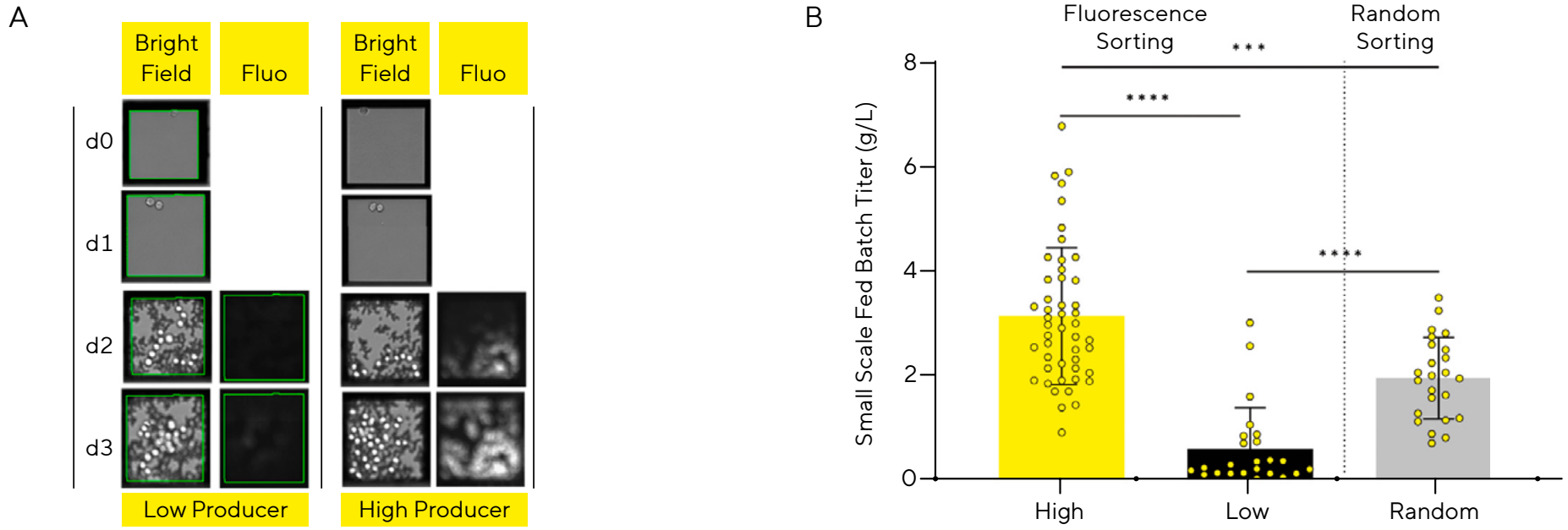


Figure 5: *Confirmation of Performance Prediction and Clone Selection.*

(A) CellCelector micrographs following a single nanowell during CLD over 4 days post single cell seeding.

(B) Box plot comparing the titers of clones selected with the CellCelector based on fluorescence level compared to random CellCelector sorting as a control and expanded in small scale fed batch culture in 96-well plates.

Chapters

Characterizing a Range of Product Producing Clones Using the CellCelector

When producing new biotherapeutics, such as mAbs, variations of the same product may differ in their toxicity to cells, which can impact outgrowth. They may also differ in complexity, size or post-translational modifications, which can impact yield. For these reasons, it is important to measure yield and outgrowth of clones producing different products.

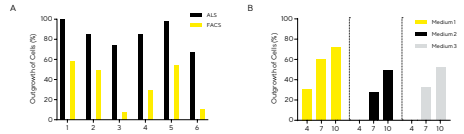


Figure 3: Clone Outgrowth Comparisons Exemplify the Use of CellCelector as a Solution for Cell Line Development.

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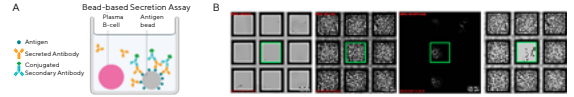


Figure 4: Clone Product Analysis Implementing a CellCelector Nanowell-Based Secretion Assay.

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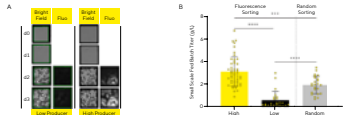


Figure 5: Confirmation of Performance Prediction and Clone Selection.

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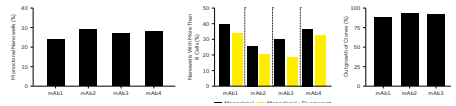


Figure 6: Confirmation of Outgrowth in Nanowells During CLD.

Click to Expand

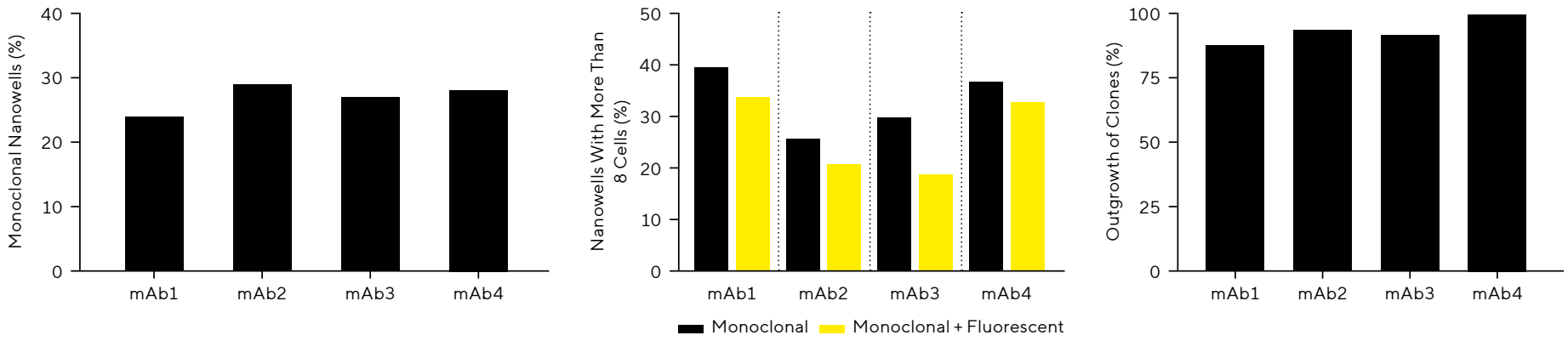


Figure 6: *Confirmation of Outgrowth in Nanowells During CLD.*

Analysis of different metrics for clone outgrowth of cell lines producing 4 different mAb products picked using the CellCelector.
(A) Monoclonality assessment at Day 0. Bar graph comparing the percentage of monoclonal nanowells for a range of clones producing different mAb products.

(B) Comparison of cell growth and fluorescence of each mAb product in nanowells at Day 4.

(C) Clone outgrowth of transferred clones in 384-well plates 11 Days after single cell cloning.

Conclusion

Traditional CLD methods can be very time consuming and resource intensive, with low success rates. Using the CellCelector during key points in the CLD workflow, we have demonstrated how this system can reduce the time and resources required to successfully produce high-quality clones with guaranteed monoclonal lineage. The key advantages of using the CellCelector platform over conv entional methods are:

- Increased levels of clone outgrowth and viability, with the ability to optimize culture conditions within the same platform
- The ability to predict clone production capacity and yield at very early stages of CLD, informing decision making prior to expensive expansion stages
- Streamlined monitoring of multiple clone types to compare potential product yield, cell health and outgrowth simultaneously, saving time and resources
- High efficiency, a small number of cells and consumables are required to produce large numbers of clones with guaranteed, tracked monoclonality

The data presented in this application note highlights the advantages of the CellCelectorfrom Sartorius as a platform for CLD, taking advantage of automation, advanced single cell picking and seeding and the reduction in resource use exemplified by the nanowell system.

Integrated Automation Trends in Cell Line Development and 3D Biology:

Improving Consistency, Throughput, and Early Decision-Making

Darius Wilson, PhD – Manager – Product Management – Sartorius

Modern biologics and cell therapy programs increasingly rely on workflows that can generate high-quality cell lines and physiologically relevant 3D models with both speed and reproducibility. Yet many laboratories continue to experience bottlenecks rooted in manual processes, heterogeneous culture formats, and fragmented instrumentation. These challenges introduce variability, constrain throughput, and make it difficult to compare results across experiments or sites.

To address these limitations, research groups are adopting automation strategies that unify single cell isolation, imaging, and clonal assessment into a structured, traceable process. Rather than treating each workflow step as an isolated task, integrated approaches are emerging that allow investigators to monitor cell behaviour from the initial isolation event through early productivity measurements or organoid maturation.

Click to Expand

- Evolving Needs in Cell LineDevelopment
- Early Productivity Screening as a Decision-Support Tool Expectation
- Parallel Challenges in 3D Biology
- Toward More Connected, Data-Rich Workflows



"With CellCelector, your team moves from single cell → validated clone → production ready line through one tightly integrated, automation driven workflow."

"Faster. Smarter. More Reliable."

Dr. Darius Wilson
Head of Product Management,
Cell Selection & Retrieval, Sartorius Lab Product & Services

Evolving Needs in Cell Line Development

1. Throughput Requirements Are Increasing
Production programs often require screening tens of thousands of single cell candidates. Traditional limiting dilution or manual selection techniques rarely scale to such volumes without compromising monoclonality assurance. Many teams now look to plate formats that support higher well densities and clearer isolation boundaries to reduce the likelihood of mixed colonies.

2. Demonstrating Monoclonality Remains a Regulatory Expectation
Regulators typically expect image-based evidence tracing a production clone back to a verified single cell. Consistency in imaging, documentation, and well tracking therefore plays a critical role in downstream compliance.

3. Early Insight into Cell Behaviour Reduces Development Timelines
Selecting clones solely based on outgrowth or morphology often delays the identification of high producing clones. Increasingly, researchers incorporate early fluorescencebased productivity assays or secretion measurements to help prioritize higher-value candidates sooner. A recent case study from a major pharmaceutical company illustrates this shift. Researchers compared standard culture plates with Next Generation Nanowell designs and observed several practical advantages: a higher number of detected nanowells, a larger proportion of verified monoclonal wells, and more robust outgrowth visible as early as Day 3. By Day 4, the number of clones considered “ready to pick” had nearly doubled in some conditions, significantly expanding the candidate pool for subsequent screening (Table 1).

Cell Types	Seeding Density (cells/ml)	Plate Type	Detacted Nanowell in One Macrowell	Empty Wells (N)	Monoclonal Wells (N)	Conflict Wells (N,%)	Clone Growth (N,%)	Ready to Pick (N,%)
CHO K1	1000	Standard	2170	1411	552	4 (0.28%)	268 (48.9%)	42 (7.6%)
CHO K1	3000	Standard	2170	719	704	3 (0.42%)	384 (54.5%)	138 (19.6%)
CHO K1	1000	Next Generation	2868	2038	652	3 (0.15%)	386 (59.2%)	106 (16.3%)
CHO K1	3000	Next Generation	2862	1140	980	4 (0.35%)	695 (70.9%)	480 (49.0%)
HEK 293	1000	Standard	2177	1631	259	0 (0.00%)	21 (8.1%)	3 (1.2%)
HEK 293	3000	Standard	2169	1024	404	1 (0.1%)	163 (40.3%)	54 (13.4%)
HEK 293	1000	Next Generation	2870	2356	287	0 (0.00%)	57 (19.9%)	28 (9.8%)

Table 1: *CHO K1 and HEK 293 monoclonality and growth comparison between the Standard and Next Generation Nanowell plates.*

Early Productivity Screening as a Decision-Support Tool

High sensitivity fluorescence imaging modes and automated calibration workflows are becoming increasingly relevant as teams work with difficult to express constructs or low secretion clones. These capabilities support data collection at earlier time points, often within three to four days post seeding, providing researchers with actionable patterns before committing resources to scale up.d nearly doubled in some conditions, significantly expanding the candidate pool for subsequent screening (Table 1).

The ability to detect subtle differences in expression early— rather than waiting for extended expansion phases—can meaningfully reduce resource use and help laboratories focus on clones with higher development potential.

Parallel Challenges in 3D Biology

Similar pressures exist in 3D model development. Spheroids and organoids offer more physiologically relevant contexts for toxicology, disease modelling, and functional studies, but can be difficult to generate reproducibly. Key challenges include reliable singlecell seeding, consistent monitoring of early growth, and objective selection of structures that meet specific size or morphological criteria.

Studies integrating automated selection with livecell imaging have shown that these approaches can help standardize spheroid formation, monitor growth trajectories, and support the isolation of organoids based on lineage-specific markers. For many labs, this has enabled the creation of more uniform and assay-ready 3D systems, improving both experimental reproducibility and biological relevance.

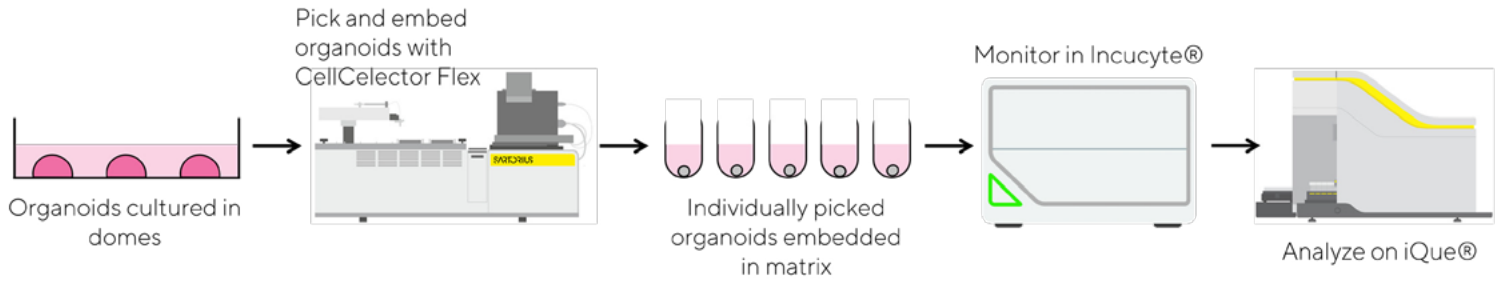
App Note: Integrated iPSC Spheroid & Organoid Workflow

Toward More Connected, Data-Rich Workflows

Across both cell line development and 3D biology, a common pattern is emerging, scientists benefit when previously separate steps—single-cell isolation, imaging, clone tracking, productivity measurement, and organoid selection—are combined into cohesive, data-driven workflows. Such integration supports:

- Reduced operator-dependent variability
- Clear traceability from single cell to validated clone or organoid
- Earlier decision-making grounded in quantitative measurements

Greater scalability for high-throughput programs As pressures on speed, reproducibility, and regulatory transparency continue to rise, workflows that consolidate these capabilities are becoming increasingly valuable. Whether the goal is to establish a production-ready cell line or to generate a physiologically meaningful 3D model, integrated automation strategies provide researchers with a practical path toward more reliable and efficient outcomes.



Case Study: CellCelector Next Generation CLD Nanowell Plates

CellCelector Next Generation CLD Nanowell plates enable the high-throughput screening, identification and transfer of up to 85,000 candidate clones from a single plate, thereby offering a cost-effective alternative to conventional limiting dilution, FACS and other single cell screening and cloning methods. Nanowell plates are available in either ultra-low attachment (for CHO, HEK and hybridoma) or plasma (for IPSC and cancer cell) coatings to provide optimal outgrowth conditions.

CellCelector Nanowell plates provide elegant solutions to common cell line development challenges, such as:

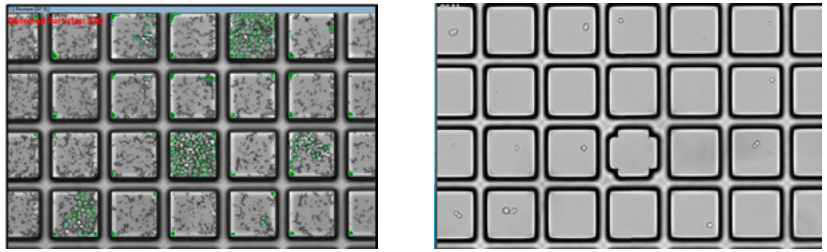
Challenges:
- Throughput: Increasing the number of monoclonal wells during a scan to identify potential candidate clones
- Regulatory Assurance: Image-based monoclonality assurance
- Clone Outgrowth: Ensuring high clone outgrowth from a monoclonal cell, especially within difficult to culture cells

Provided Solution:
CellCelector Next Generation Nanowell Plates:
- Significantly increase the number of available wells within a plate
- Higher walls decrease the number of conflict wells observed during a scan
- Demonstrate significantly higher clone outgrowth across a range of different cell types

Click to Expand

Background Information +
Results +
Comparison Methods, Analysis and Results: Better Clone Outgrowth +
Comparison Methods, Analysis and Results: Thinner Walls and Reference Wells +

40% more monoclonal wells
<0.1% conflict wells
>3 times better clone outgrowth



Case Profile

Company Type:
Large pharmaceutical company developing medicines and vaccines in core therapeutic areas.

Application Description:
Identification and characterization of different cell lines within the cell line development workflow to identify candidate clones for upstream processing.

Background Information

Cell Types	CHO K1 and HEK 293-6E cell lines maintained in suspension
Plate Type	Standard and Next Generation ultra-low attachment coated Nanowell plates
Seeding Density	Both cell lines were seeded at 1000 and 3000 cells per ml, respectively, across 2 macrowells
Detected Nanowells	Average number of Nanowells detected per macrowell, using a 1000 µm Nanowell border distance
Empty Nanowells	Average number of empty Nanowells detected per macrowell
Monoclonal Nanowells	Average number of Nanowells detected per macrowell containing an image verified single cell
Conflict Wells	Average number of empty Nanowells on D0 where one or more cells were found on D4
Clone Outgrowth	Average number of monoclonal Nanowells containing ≥3 cells on D4
Ready to Pick	Average number of monoclonal Nanowells containing a clone consisting of ≥8 cells on D4
Analysis	Wells were scanned using a 10x objective in brightfield on immediately after seeding (day 0) and on day 4 and analysed using identical settings across all conditions

Results

When measured in one single Macrowell, results betweenthe Standard and Next Generation Nanowell plates highlight a significant increase in the number of detected Nanowells using the Next Generation Nanowell plates (Table 1). Accordingly, the number of wells containing a single, monoclonal cell were also significantly increased. Despite the increase in detected wells, the number of conflict wells remained constant, or was even slightly decreased. Results were similar for both CHO K1 and HEK 293 cells, although the number of HEK 293 conflict wells detected was negligible. To calculate the number of monoclonal wells within a fully utilised 24 Macrowell Next Generation Nanowell plate, all results in Table 1 should be multiplied by a factor of 24.

Importantly, clone outgrowth was significantly higher for both the CHO K1 and HEK 293 cells on day 3, as well as those classified as "ready to pick" on day 4.

			Day 0 (Day of Seeding)				Day 4	
Cell Type	**Seeding Density (cells/ml**	**Plate Type**	**Detected Nanowells in One Macrowell**	**Empty Wells (N)**	**Monoclonal Wells (N)**	**Conflict Wells (N,%)**	**Clone Growth (N,%)**	**Ready to Pick (N,%)**
CHO K1	1000	Standard	2170	1411	552	4 (0.28%)	268 (48.9%)	42 (7.6%)
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HEK 293	3000	Standard	2169	1024	404	1 (0.1%)	163 (40.3%)	54 (13.4%)
HEK 293	1000	Next Generation	2870	2356	287	0 (0.0%)	57 (19.9%)	28 (9.8%)
HEK 293	3000	Next Generation	2874	1616	544	1 (0.06%)	230 (42.3%)	141 (25.6%)

Table 1: *CHO K1 and HEK 293 monoclonality and growth comparison between the Standard and Next Generation Nanowell plates.*

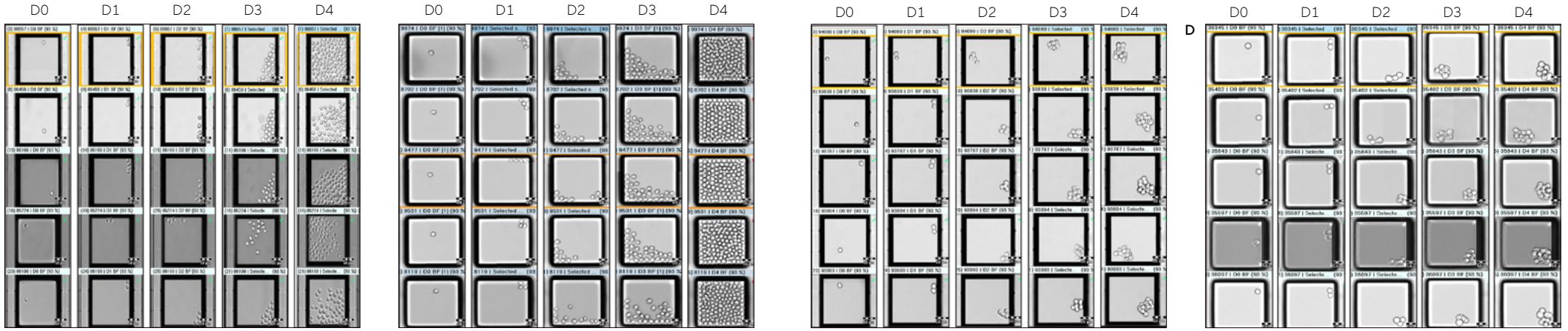
Comparison Methods, Analysis and Results: Better Clone Outgrowth

A Standard CellCelector Nanowell Plates

B Next Generation CellCelector Nanowell Plates

C Standard CellCelector Nanowell Plates

D Next Generation CellCelector Nanowell Plates



Comparison of Standard Nanowell CellCelector plates and Next Generation CellCelector plates to highlight differences in (A and B) CHO K1 and (C and D) HEK 293 clone growth between D0 and D4 after seeding at 3000 cells per ml. Results show significantly higher growth using the Next Generation CellCelector CLD plates on D4.

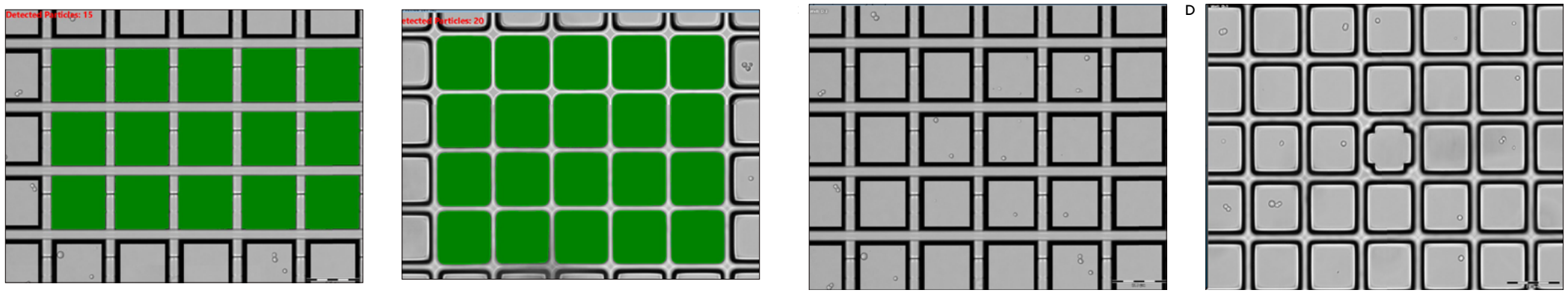
Comparison Methods, Analysis and Results: Thinner Walls and Reference Wells

A Standard CellCelector Nanowell Plates

B Next Generation CellCelector Nanowell Plates

C Standard CellCelector Nanowell Plates

D Next Generation CellCelector Nanowell Plates



Comparison of Standard Nanowell CellCelector plates and Next Generation CellCelector plates to highlight differences in (A and B) plate wall structure, and (C and D) presence of reference wells.

Four Undeniable Benefits of Automated Single-Cell Isolation in Cell Line Development

Biologics, also known as biopharmaceuticals, stand at the forefront of innovation in modern medicine.[1] In recent years, biologics have accounted for almost 50% of new drug approvals, including a range of vaccines, gene therapies, and recombinant proteins. Significantly, over the past four years, 84% of the biologics approved for therapeutic use have been produced using mammalian cells, highlighting their vital role in this sector.

Navigating Bottlenecks in Cell Line Development

The development of stable and robust cell lines is the cornerstone of biologics production. However, this process presents numerous logistical and technical challenges (Figure 7). Biologics produced from cell-based systems are inherently more complicated compared to chemically synthesized drugs, leading to higher risks of environmental impact and microbial contamination. Consequently, protocols in cell line development must be carefully crafted to ensure optimal cultivation conditions.[1,5] A key challenge in this process is the isolation of single cells, crucial for maintaining the consistency and efficacy of biologics. Traditional manual methods used in this stage are not only labor-intensive, but also prone to inaccuracies, often resulting in costly delays. Given the complexity of developing mammalian cell lines, typically a 12–18 month process fraught with bottlenecks, the integration of automation and high-throughput technologies is becoming increasingly essential for streamlining processes like single-cell isolation.[4]

Automated, high-throughput systems accelerate development timelines by enabling faster and more informed decision-making. Moreover, an often-overlooked benefit is the enhancement of documentation support, which is especially important in an industry governed by stringent regulatory standards. Automated systems provide robust documentation capabilities, ensuring traceability of each step, from clone selection to final cell banking.



Figure 7: The stages of cell line development.[4]

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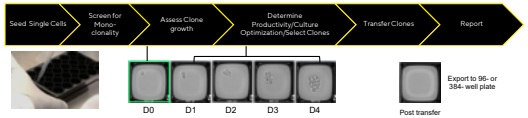


Figure 8: CellCelector Nanowell cell line development workflow.

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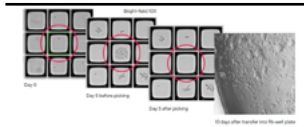


Figure 9: Automated, image-verified tracking of single clones.

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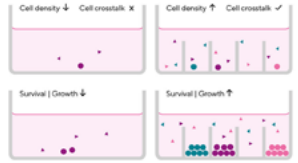


Figure 10: Cellular crosstalk in dilution-based methods vs. the nanowell-based method.

Click to Expand

1. Speed Up Clone Selection Through Automation

Transfecting an expression vector into a cell line results in a heterogenous population as not all cells will successfully express the gene of interest. Therefore, bulk analysis only provides averaged data and results in the loss of valuable information. This is not conducive to the production of biologics, which requires monocultures to achieve maximum yield.

Single-cell isolation is used to achieve homogenous populations, however traditional methods of single-cell isolation, such as limiting dilution and fluorescenceactivated cell sorting (FACS), are not only labor-intensive, but also prone to causing cell stress and reduced viability.[6,7] The CellCelector represents a paradigm shift, automating the tedious and often imprecise task of screening, selecting, and isolating single cells from a heterogeneous population.

High-throughput nanowell-based image-verified cloning technology (HT-NIC) is a new method developed on the CellCelector platform that allows for the fast generation of clones in just one cloning round (Figure 8). This method utilizes CellCelector nanowell plates, which feature thousands of nanowells at the bottom of every well. In the case of a 24-well plate, there are 3,000 nanowells per well, amounting to a total of 72,000 nanowells per plate. This design ensures that cells captured within the nanowells are efficiently separated from one another.





Figure 7: The stages of cell line development.[4]

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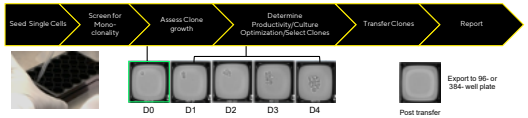


Figure 8: CellCelector Nanowell cell line development workflow.

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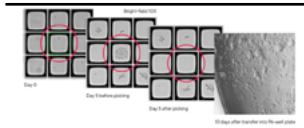


Figure 9: Automated, image-verified tracking of single clones.

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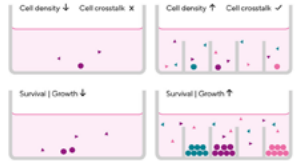


Figure 10: Cellular crosstalk in dilution-based methods vs. the nanowell-based method.

Click to Expand

2. Ensure Monoclonality with Advanced Technology

Monoclonality is a critical requirement in the production of safe and effective biologics. Traditional monoclonal assessment methods are typically manual and retrospective, lacking sufficient efficiency and accuracy. The CellCelector, however, ntroduces a more reliable method with 100% proof of monoclonality, significantly accelerating the initial stages of biologics production.

This is due to the innovative nanowell plates that allow for the development of a large number of viable clones from a single plate. After monoclonality has been documented, cells are incubated for 3–6 days, resulting in 20–75 cells per clone. The nanowell plate is then scanned again and monoclonal, viable clones are automatically selected and transferred into 96- or 384-well plates for further analysis and upscaling (Figure 9).

Each cell seeded into these nanowells is given a unique ID and is continuously tracked through growth, assessment, and colony picking. Automated scanning of the nanowell plates for viability and monoclonal status, coupled with the automatic picking and transferring of ideal clones for further expansion, ensures a streamlined and accurate process.



Figure 7: The stages of cell line development.[4]

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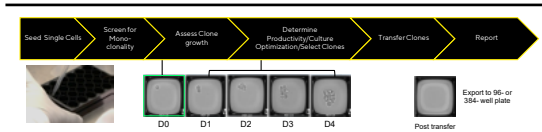


Figure 8: CellCelector Nanowell cell line development workflow.

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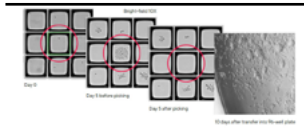


Figure 9: Automated, image-verified tracking of single clones.

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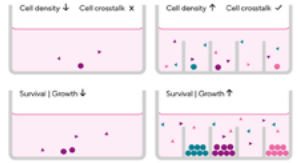


Figure 10: Cellular crosstalk in dilution-based methods vs. the nanowell-based method.

Click to Expand

3. Enhance Cellular Growth with Nanowell Plates

Some single-cell isolation methods can negatively impact the growth, viability, and even the delicate programming of cells. For example, dilution-based protocols remove growth-promoting factors from the cell culture media, leading to cell stress. While FACS offers a faster workflow for isolating subpopulations, the process has been shown to cause cell damage and reduce viability.

The CellCelector's nanowell plates are designed to promote optimal cellular growth and health, addressing this important limitation. Unlike traditional cell culture plates, the nanowells allow physically isolated single cells to engage in chemical communication through the shared medium (Figure 10). This feature is particularly beneficial for difficult-to-grow cell types, as it maintains the necessary conditions for cell survival and growth, while ensuring monoclonality.

The gentle picking and seeding process of the CellCelector further maintains the integrity of the clones, leading to industry-high levels of cellular outgrowth following isolation.



Figure 7: The stages of cell line development.[4]

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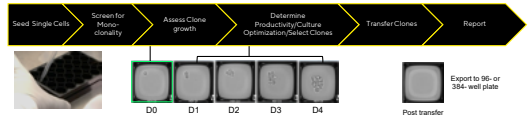


Figure 8: CellCelector Nanowell cell line development workflow.

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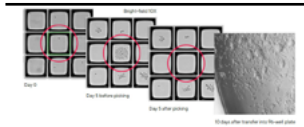


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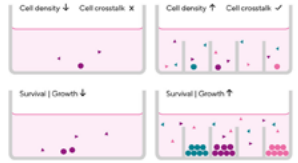


Figure 10: Cellular crosstalk in dilution-based methods vs. the nanowell-based method.

Click to Expand

4. Simplify Documentation and Compliance

In the highly regulated field of biomanufacturing, maintaining accurate and comprehensive records is essential. The CellCelector Flex not only optimizes the practical aspects of cell line development,but also provides detailed workflow documentation.

The system captures high-quality, real-time images before and after each picking event, ensuring thorough monitoring and quality control of the cell selection process. Each cell or clone is assigned a unique ID, enabling precise tracking from the source to the destination well. By automating and simplifying the documentation process, the CellCelector significantly reduces the potential for human error and increases efficiency, making it an invaluable asset in the cell line development process.



Figure 7: The stages of cell line development.[4]

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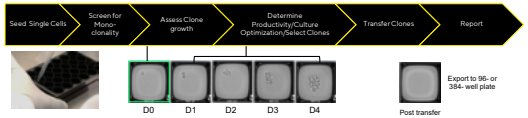


Figure 8: CellCelector Nanowell cell line development workflow.

Click to Expand

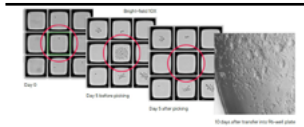


Figure 9: Automated, image-verified tracking of single clones.

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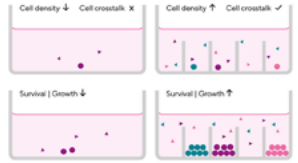


Figure 10: Cellular crosstalk in dilution-based methods vs. the nanowell-based method.

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Figure 7: *The stages of cell line development.*[4]

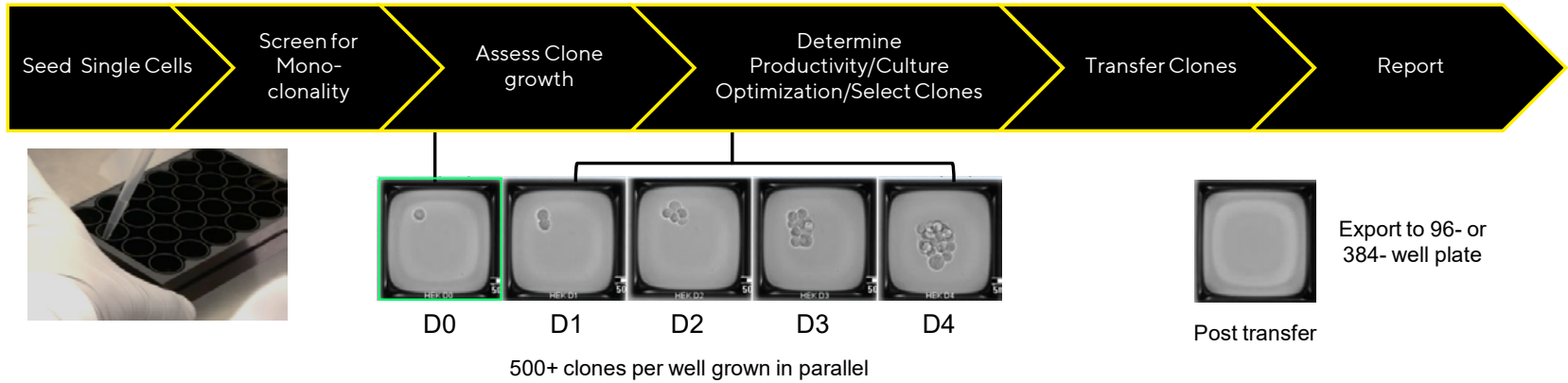


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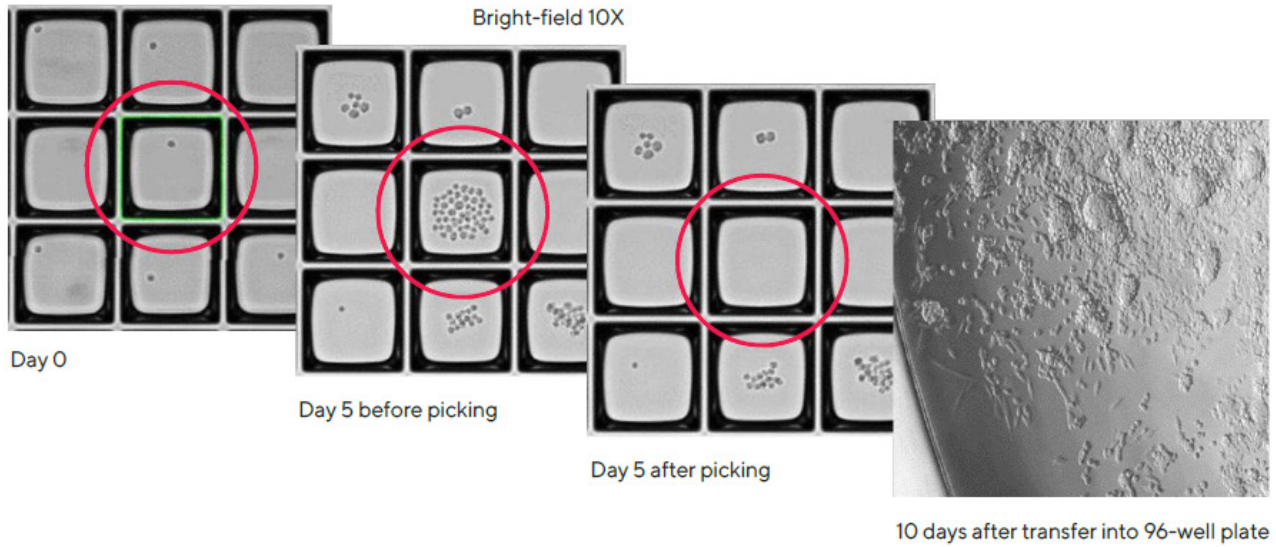


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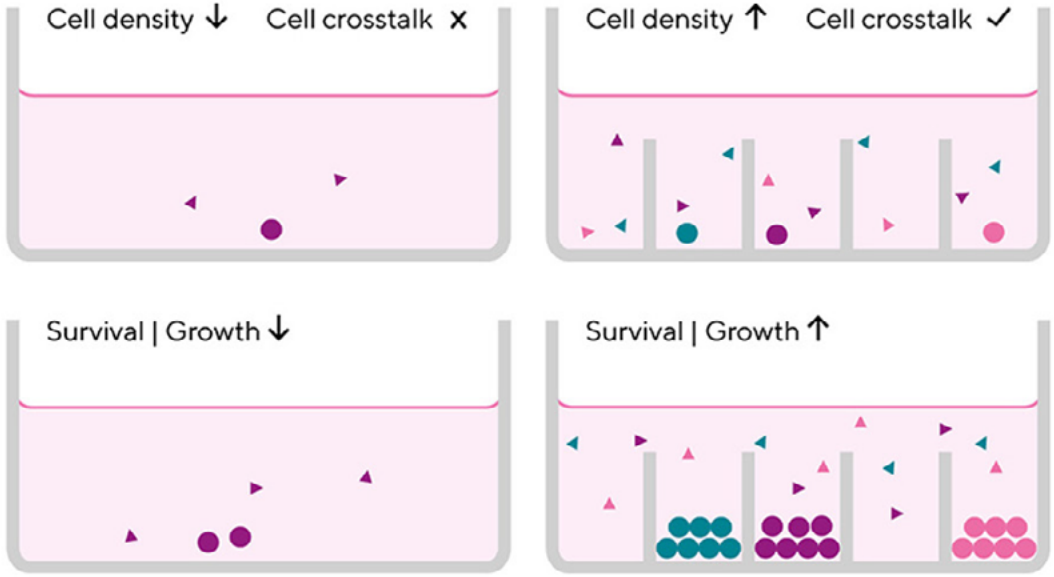


Figure 10: *Cellular crosstalk in dilution-based methods vs. the nanowell-based method.*

Conclusion

The CellCelector addresses important needs in cell line development, offering significant advancements in clone selection, monoclonality assurance, cellular growth, documentation, and process efficiency. In addition to the CellCelector, Sartorius offers a suite of other instrument platforms that play supportive roles in the comprehensive cell line development process. Notably, then iQue® HTS by Cytometry (for multiplexed analysis), the Octet® BLI Label-Free Detection System (for real-time protein quantitation), the Ambr® 15 Cell Culture (for optimizing culture conditions), and the Fill-It (for automated cryovial filling) complement the capabilities of the CellCelector Flex, together creating a robust and efficient workflow for biologics production.



Figure 7: The stages of cell line development.[4]

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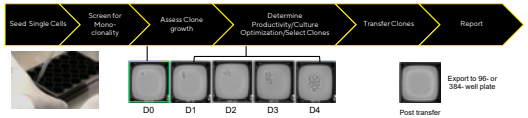


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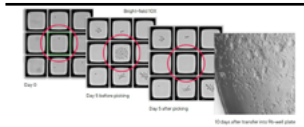


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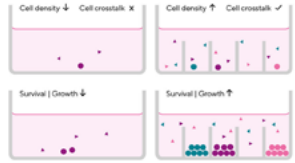


Figure 10: Cellular crosstalk in dilution-based methods vs. the nanowell-based method.

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Figure 7: The stages of cell line development.[4]

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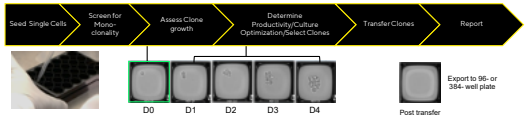


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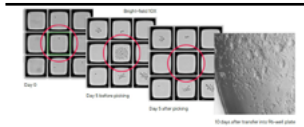


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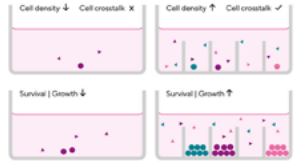
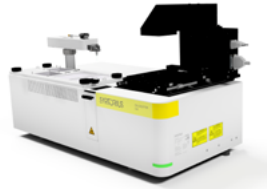


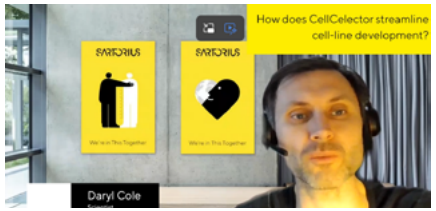
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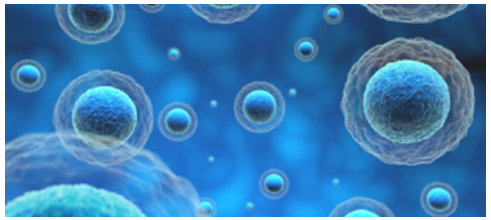
Resources



Video: CellCelector: Single-Cell, Colony or Organoid Selection and Retrieval



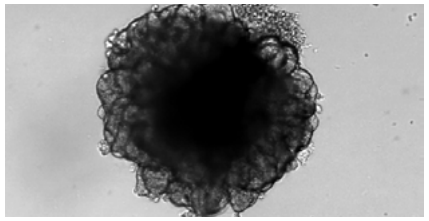
Video: Science Corner: How Does CellCelector Streamline Cell-Line Development?



On-Demand Webinar: Optimizing Clone Selection in Cell Line Development



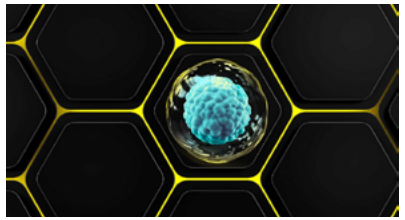
eBook: Analyzing Critical Quality Attributes for Efficient and Rapid Cell Line Development



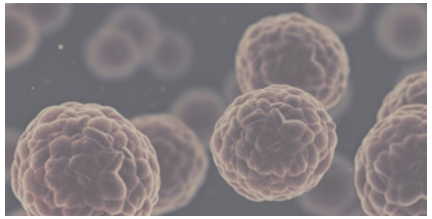
Webinar: Advanced Cell Model Workflows and CellCelector Flex: Flexible Organoid Picking



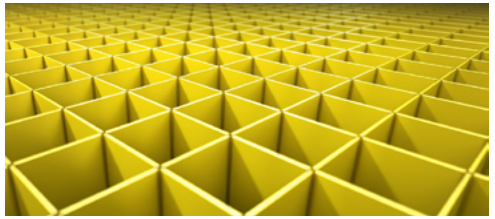
Application Note: Automated Monoclonality Screening and HEK Cell Line Development



On Demand Webinar: Novel Single-Cell Cloning Technology in Cell Line Development



Case Study: Monoclonality Assurance with Nanowell Cell Cloning on HEK293SF-3F6 Cell Lines



Webinar: Advanced Predictive Modeling in Cell Line Development

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