SARTORIUS

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

Case Profile

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

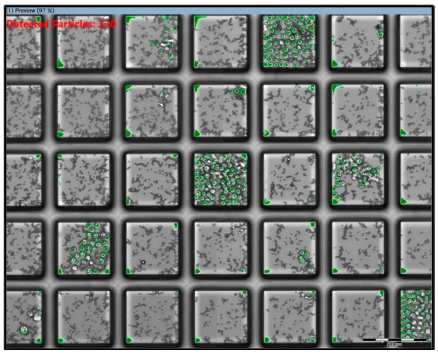
Application Description:
Identification and characterization of different cell lines within the cell line development workflow to identify candidate clones for upstream processing.

For further information, visit
https://www.sartorius.com/en/products/cell-selection-and-retrieval

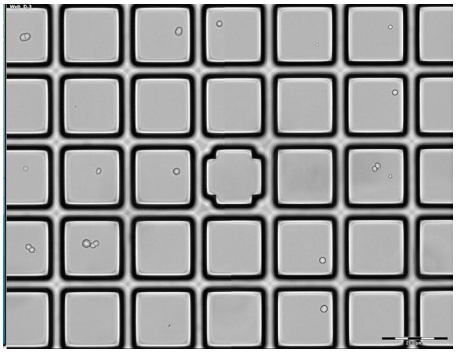
40% more monoclonal wells

<0.1% conflict wells

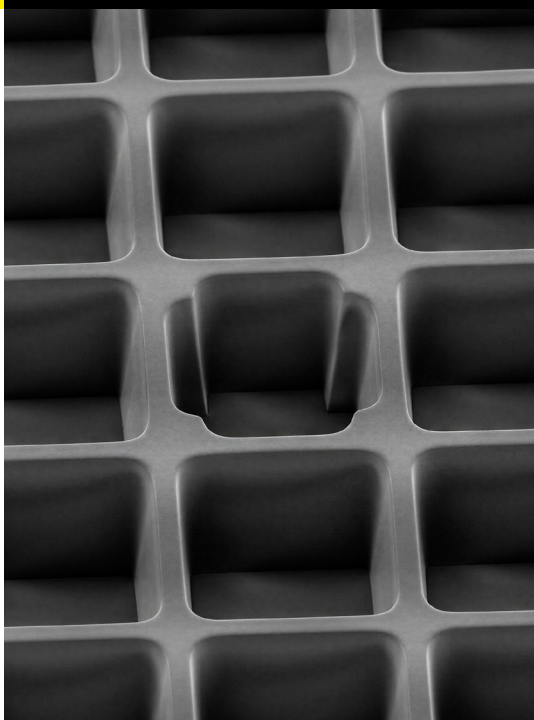
>3 times better clone outgrowth



Optimized clone productivity measurements



Reference wells for sequential imaging



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 between the 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%)
CHO K1	3000	Standard	2176	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 (0.0%)	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 (0.0%)	57 (19.9%)	28 (9.8%)
HEK 293	3000	Next Generation	2874	1616	544	1 (0.06%)	230 (42.3%)	141 (25.6%)

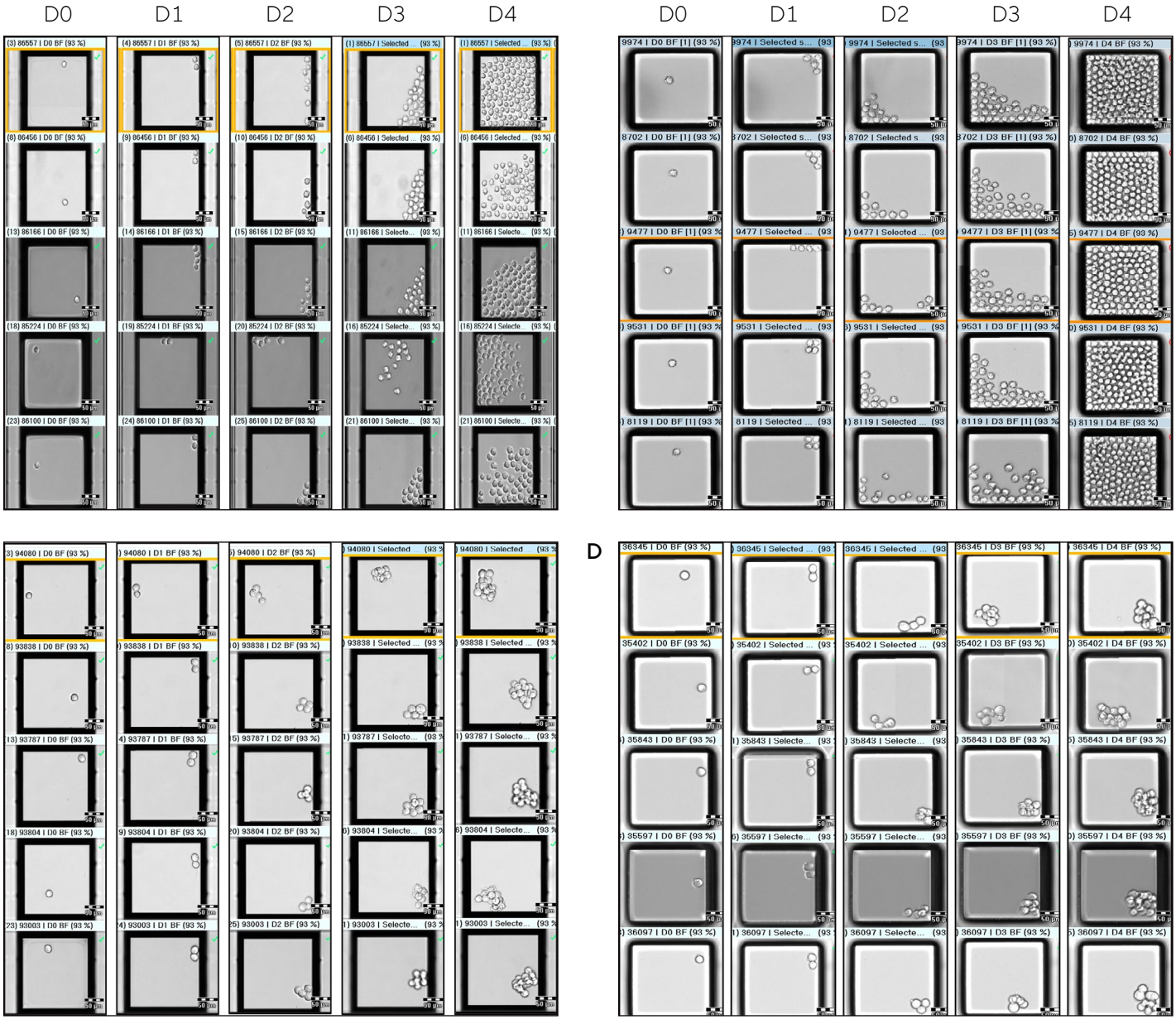
Table 1. CHO K1 and HEK 293 monoclonality and growth comparison between the Standard and Next Generation Nanowell plates.

Next Generation CellCelector CLD Nanowell Plate Comparison Methods, Analysis and Results

Better Clone Outgrowth

A Standard CellCelector Nanowell Plates

B Next Generation CellCelector Nanowell Plates



C

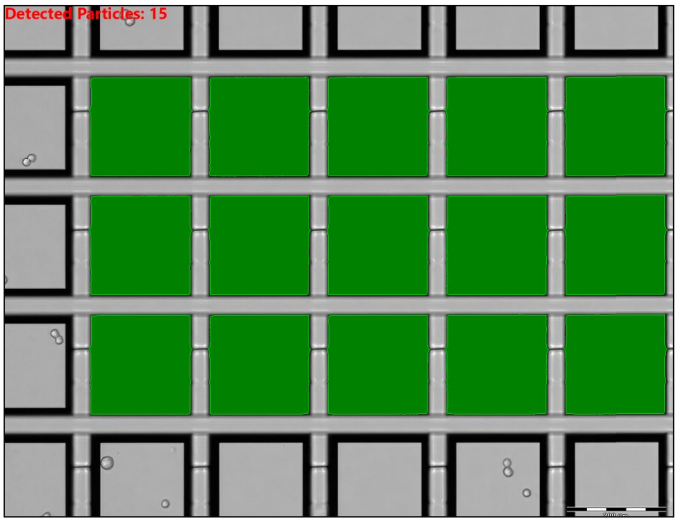
D

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.

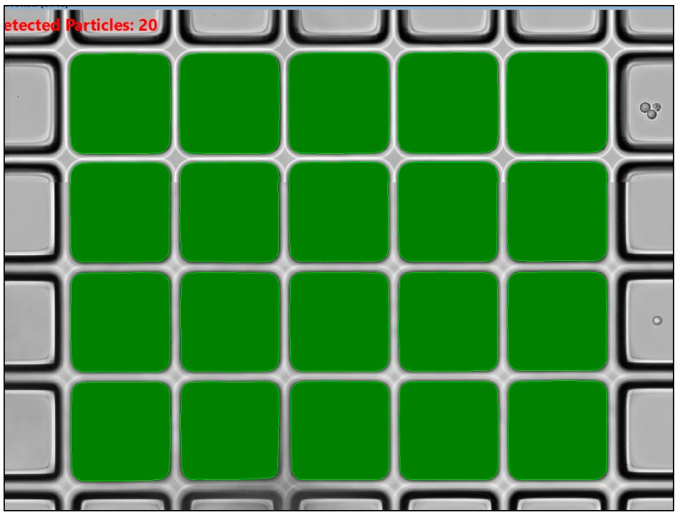
Next Generation CellCelector CLD Nanowell Plate Comparison Methods, Analysis and Results

Thinner Walls and Reference Wells

A Standard CellCelector Nanowell Plates



B Next Generation CellCelector Nanowell Plates



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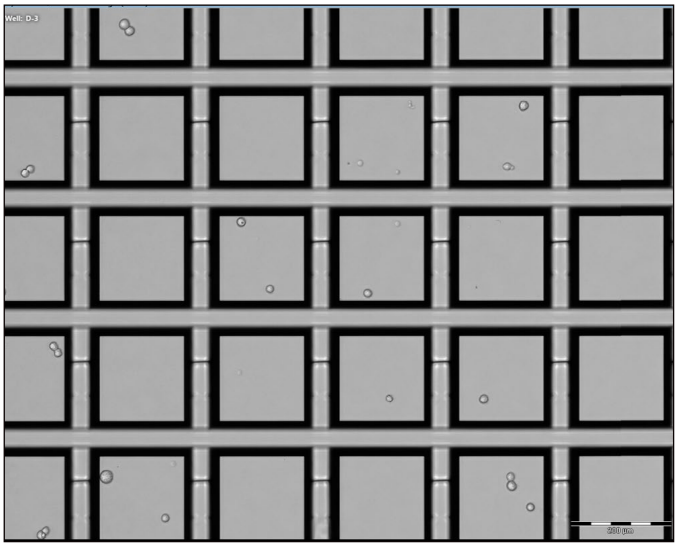

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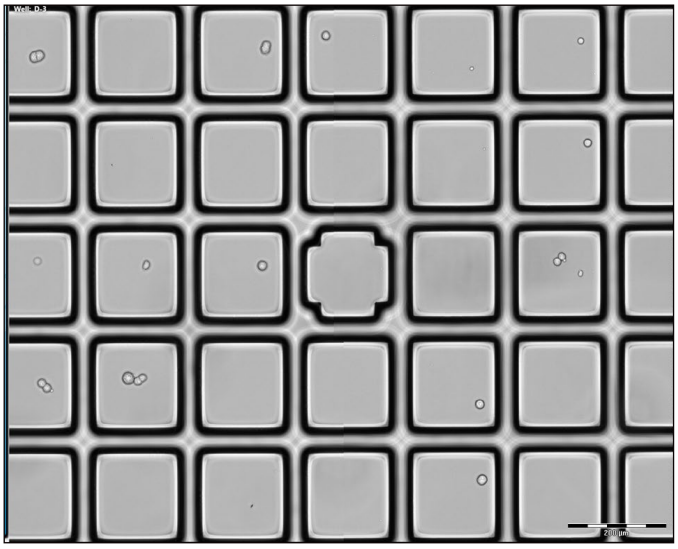

Comparison of Standard Nanowell CellCelector plates and Next Generation CellCelector plates to highlight differences in (A and B) plate wall structure, and (C and D) presence of reference wells.

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