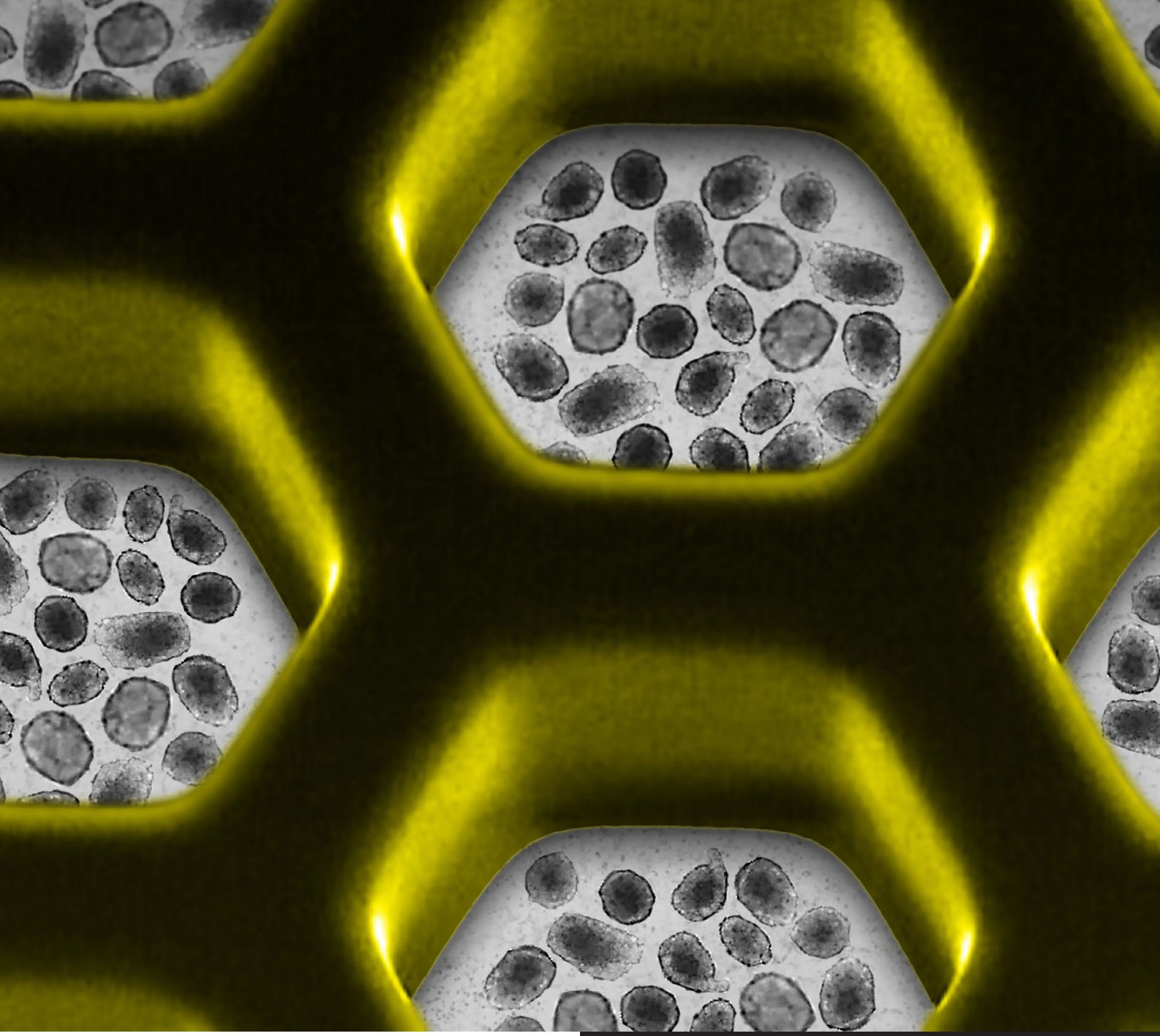


High-Throughput Nanowell-Based Image-Verified Cloning for Cell Line Development

CellCelector Flex

Simplifying Progress

SARTORIUS

High-Throughput Nanowell-Based Cloning

High-throughput nanowell-based image-verified cloning technology (HT-NIC) is a new method allowing the fast generation of clonal pharmaceutical production cell lines. Clones are generated in just one cloning round while providing robust in-process image-verified monoclonality proof.

Due to this integrated monoclonality and viability assessment of clones, as well as industry-leading outgrowth rates after clone transfer into 96- or 384-well plates, the CellCelector single cell cloning technology represents the next generation of single cell cloning approaches. It goes far beyond the traditional methods and provides a superior alternative to limiting dilution (LD), fluorescence-activated cell sorting (FACS), or single cell printing techniques. This patent-pending method has been developed and validated in collaboration with ProBioGen AG and other CellCelector Flex customers.

CellCelector Flex Specifications

High Throughput
- Analysis of thousands of clones in parallel

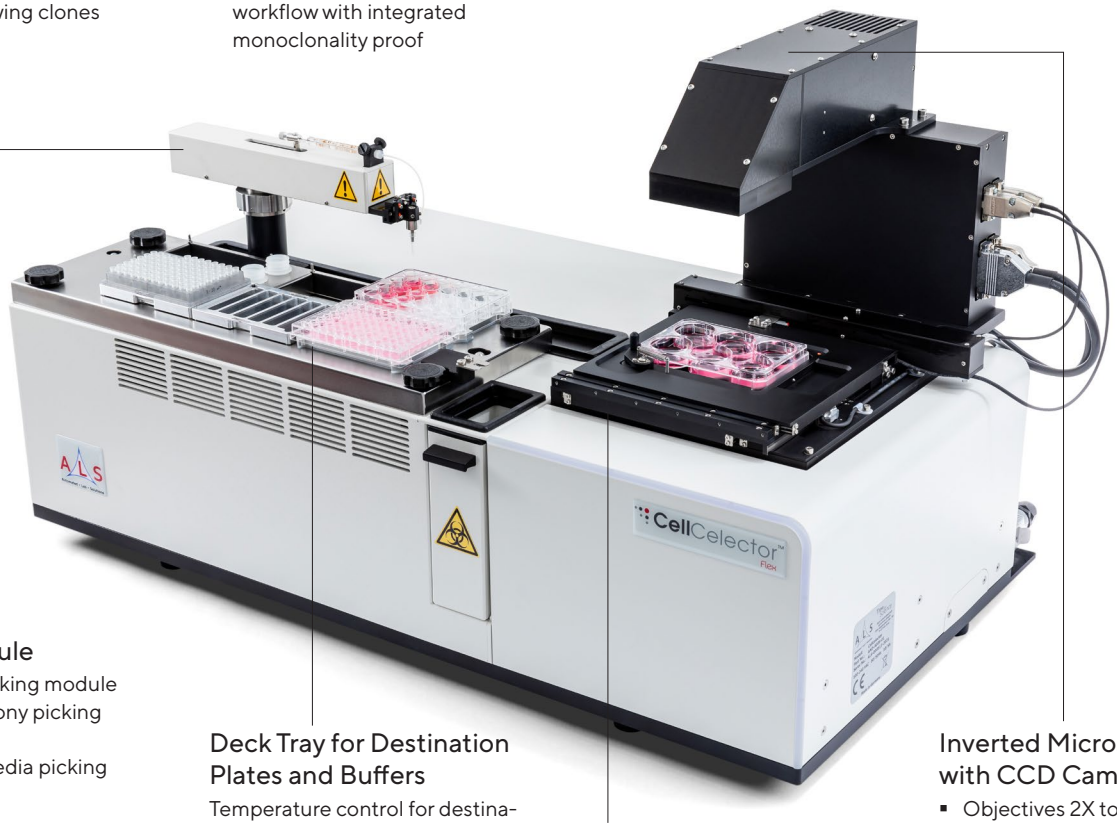
Growth Evaluation
- Selective isolation of fast- and well-growing clones

Productivity Assessment
- Targeted selection of high-producing clones

Monoclonality
- One-round single cell cloning workflow with integrated monoclonality proof

Cell Viability
- Industry-leading outgrowth rates of single cells for extremely difficult to grow cell lines

Economical Proceeding
- Considerable saving of time, consumables, media, and storage capacities



Picking Module
- Single cell picking module
- Adherent colony picking module
- Semi-solid media picking module

Deck Tray for Destination Plates and Buffers
Temperature control for destination plates (4 °C–40 °C)

Motorized High-Precision X | Y -Stage with Autofocus for Source Plates

Inverted Microscope with CCD Camera
- Objectives 2X to 40X
- Bright-field (BF)
- Phase contrast (PhC)
- Fluorescence with 6 excitation channels and up to 14 colors using compatible fluorophores

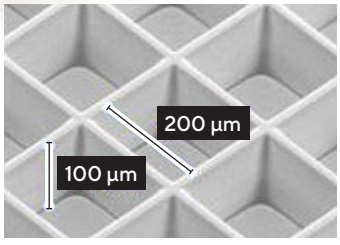
Find out more
www.sartorius.com

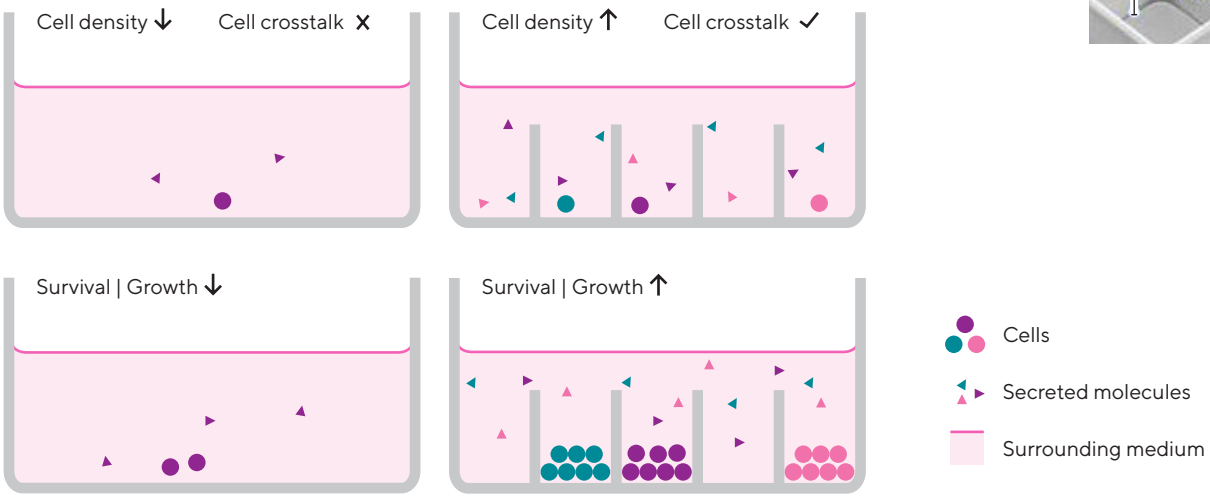
With the CellCelector Flex single cell and colony picking platform, you can now effectively assess and verify your clones before deciding which ones to choose. In less than one week, you will obtain monoclonal, viable, and productive colonies from your pool of single cells. Instead of relying on large quantities of plates to produce a winner you can now use actual data to reliably predict the future of your clones. This saves consumable and media costs, incubator space, and most of all valuable time by avoiding missteps, a second cloning round, and unnecessary procedures.

CellCelector Nanowell Cell Culture Plates

The HT-NIC method is based on the CellCelector nanowell plates. These plates are available in different formats, featuring thousands of nanowells at the bottom of each well. For a 24-well plate this results in 4,000 nanowells per well or 100,000 nanowells per plate. Cells inside the nanowells are efficiently separated from each other.

Despite the local separation, the cells in the nanowells are covered by the same medium and thereby effective cellular crosstalk can occur. All cells in the pool will contribute to the outgrowth of the cell line while maintaining their monoclonality. This leads to industry-leading outgrowth rates of single cells even for cell lines, which are extremely difficult to cultivate.

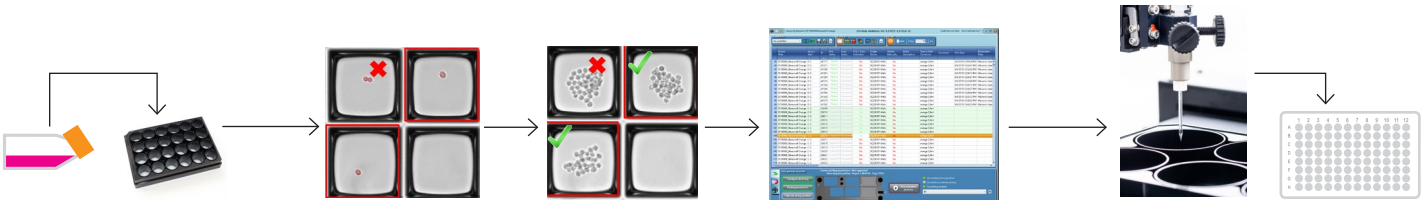




CellCelector Nanowell-Based Single Cell Cloning Workflow

Cell seeding is performed similarly to conventional cell culture plates. After seeding the cells, they are randomly captured inside the nanowells following the classic Poisson distribution. Automated scanning of the wells, followed by an automated identification of all nanowells containing a single cell, provides a robust and documented image-based monoclonality proof.

Depending on the number of cells seeded, 400–600 single cells are captured per well and can be analyzed. Seeding multiple wells allows you to start with up to ~14,000 single cells captured in individual nanowells—all within just one nanowell plate.



Cell seeding into a nanowell plate

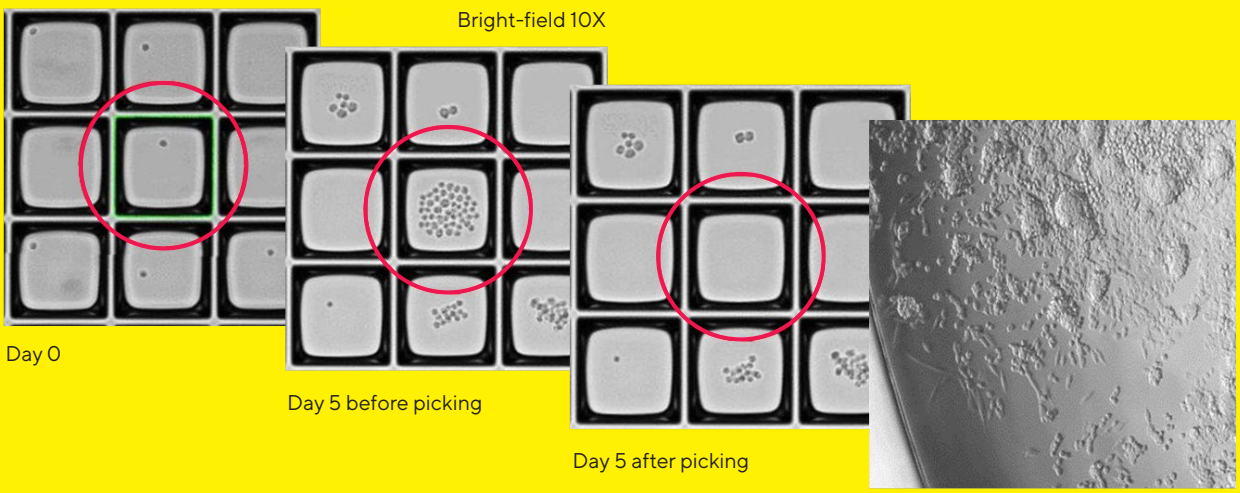
Monoclonality screening (Day 0)

Clone growth assessment (Day X)

Automated clone ranking and selection

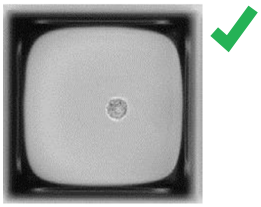
Automated transfer of selected clones for further expansion in a 96- or 384-well plate

After monoclonality has been documented, cells are incubated for 3–6 days, resulting in 20–75 cells per clone. Unlike the traditional methylcellulose-based approach, the CellCelector nanowell based method allows the colony growth in liquid media. Clones share the same media but are effectively separated from each other by the nanowell walls. After the cells have grown into single cell clones, the nanowell plate is scanned again and monoclonal, viable clones are automatically selected and transferred into 96- or 384-well plates for further analysis and upscaling.

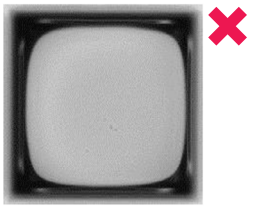


Automated Monoclonality Proof: Robust Single Cell Detection in Nanowells

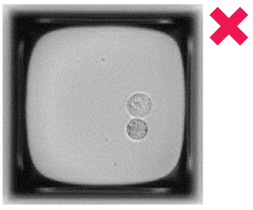
In a conventional single cell cloning workflow, single cells are seeded in 96-well plates making reliable automated single cell detection difficult as cells are often settled at the very edge of the well. Thus, the monoclonality status of a given clone at Day 0 is usually checked manually or retroactively once the clone has grown. But why search for a single cell within a well area that is more than 100 times larger than the surface occupied by a cell? With the CellCelector HT-NIC approach the cells are separated and clearly visible within 200 µm large nanowells and can therefore be identified reliably and automatically by our software. Identification is possible just after seeding and even when cells are in contact with the nanowell border.



Single cell



Empty nanowell



> 1 cell

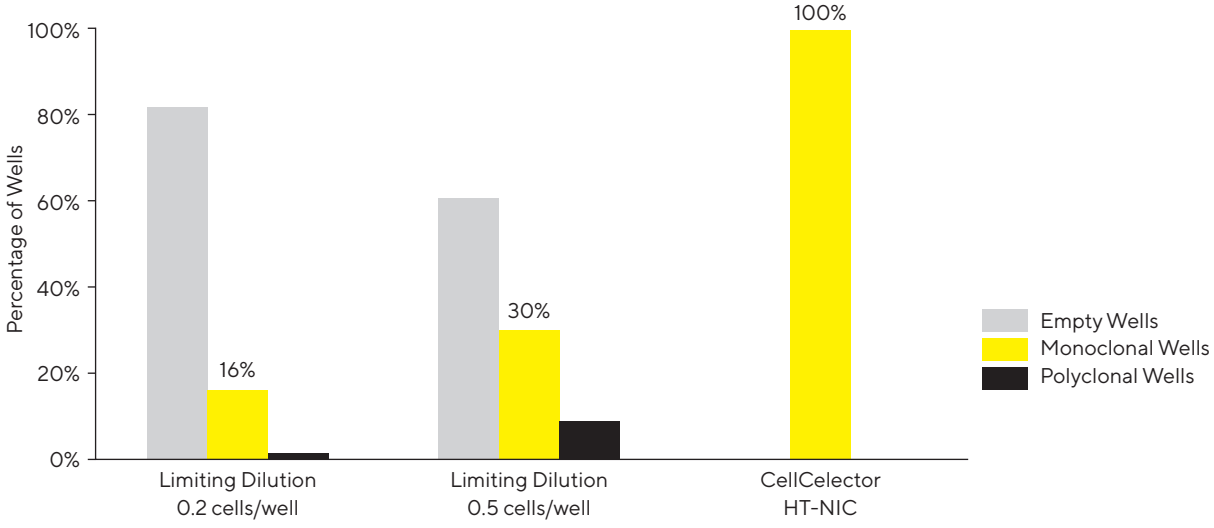
Forget Limiting Dilution, CellCelector Flex Offers 100% Monoclonality

In limiting dilution, a well-established method for single cell cloning, the monoclonality is based on statistical properties, which depend on the average cell number seeded per well according to the Poisson distribution. To limit the percentage of polyclonal wells, cell density for seeding is reduced to 0.2 cells per well. Further, only ~16% of the wells are monoclonal while the other wells stay empty. Thus, more than twenty-five 96-well plates need to be seeded to start with 400 monoclonal wells. There is the possibility to increase cell density and thereby double the number of monoclonal wells, but this comes with a high risk for polyclonal wells.

By automating identification of single cell nanowells, tracking their growth into clones, and transferring the grown clones into 96-well plates without cross-contamination, the CellCelector HT-NIC cloning method provides 100% monoclonal wells—independent of sample preparation, cell type, or the cell density used for seeding into the nanowell plate.

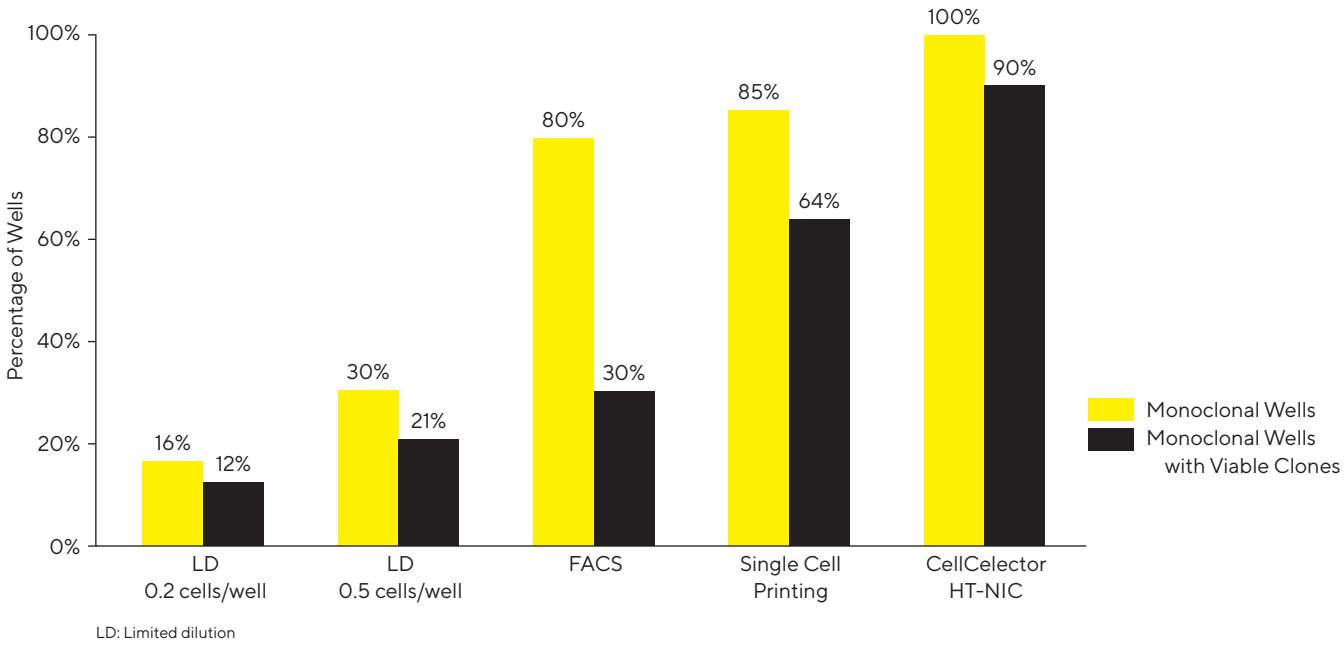
A comparison of the efficiency of obtaining monoclonal wells with the CellCelector nanowell-based cloning method or with limiting dilution is shown in the figure below.

To obtain 400 monoclonal wells using the limiting dilution method, twenty-six 96-well plates (for 0.2 cells/well seeding density) or thirteen 96-well plates (for 0.5 cells/well seeding density) need to be used. The same number of monoclonal wells is reached within just one well of the CellCelector nanowell plate using the HT-NIC method.



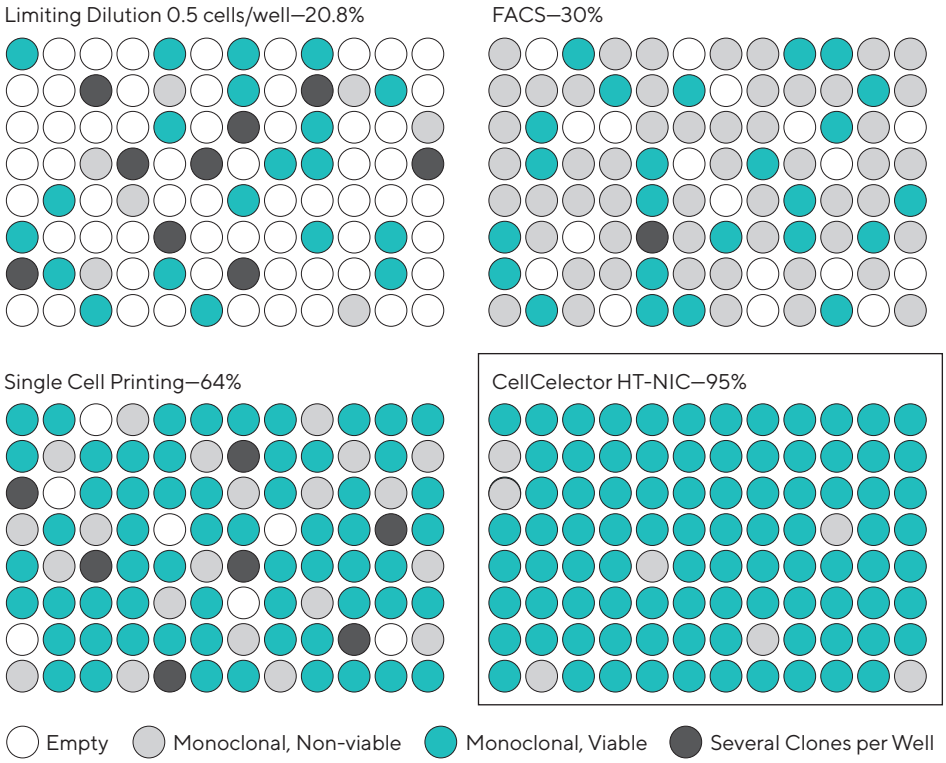
Get the Highest Percentage of Viable Single Cell Clones

The limiting dilution method leads to only 10%–20% outgrowth. Other traditional methods like FACS single cell sorting or single cell printing provide more single cells, but high shear stress usually associated with flow cytometry and the fact that the deposited single cells sit in a large volume of media on their own leads to uncertain outgrowth at rates between 30% and 64%. Also, these methods require specialized and expensive cloning media with external growth factors and are unsuitable for difficult to grow cell lines that simply won't grow from single cells.



LD: Limited dilution

In the CellCelector HT-NIC method all seeded cells share the same media and contribute to each other's growth by releasing natural growth factors without compromising clonality. Only after a clone has been proven to be monoclonal and viable will it be selected and gently transferred into a 96-well plate. Since a viable and monoclonal small clone is being transferred instead of a single cell, it will continue to grow in a large well.



Benefits of using the CellCelector Flex for High-Throughput Single-Cell Cloning

Easy to Use	▪ No complex sample preparation and no expensive consumables required ▪ No routine maintenance necessary
Extremely Versatile	▪ Precise isolation of individual single cells, clusters, single cell clones, spheroids, small organoids, embryoid bodies ▪ Primary cells and cell lines; living and fixed cells
Flexible	▪ Bright-field, phase contrast, and fluorescence imaging ▪ Automated, semi-automated, or manual cell selection for picking ▪ Any standard or custom source and destination vessels (nanowell arrays, microplates, dishes, slides, filter, chips, PCR plates | tubes
Reliable	▪ Accuracy of picking > 95% of selected specific sub-populations ▪ Automatic re-location of moving objects ▪ Possible re-picking of failed picking events ▪ Software automatically detects successful picking ▪ Automated picking quality control (picked | failed)
Gentle	▪ No influence on characteristic properties of cells ▪ Isolation of pure intact cells ready for molecular characterization or cell culture (low shear stress: < 10 seconds in the capillary) ▪ High cell integrity and outgrowth rates after picking (including up to 95% and more viability in single cell cloning applications) ▪ Automated picking quality control (picked | failed)
Downstream Compatible	▪ Low aspiration, dispensing, and buffer volumes (down to ~1 nl) ▪ Single cell PCR, NGS, WGA, RNA-Seq, genome editing, cell cloning, heterogeneity studies, etc.
Documented	▪ Complete workflow documentation compliant to GLP and GMP standards ▪ Quality control by using live-tracking and high-quality real-time images taken before and after each picking event ▪ Unique ID for each detected | picked object, tracking from source to destination well ▪ Easy export of all imaging and numeric data

Germany
Sartorius Lab Instruments
GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For additional information,
visit www.sartorius.com

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