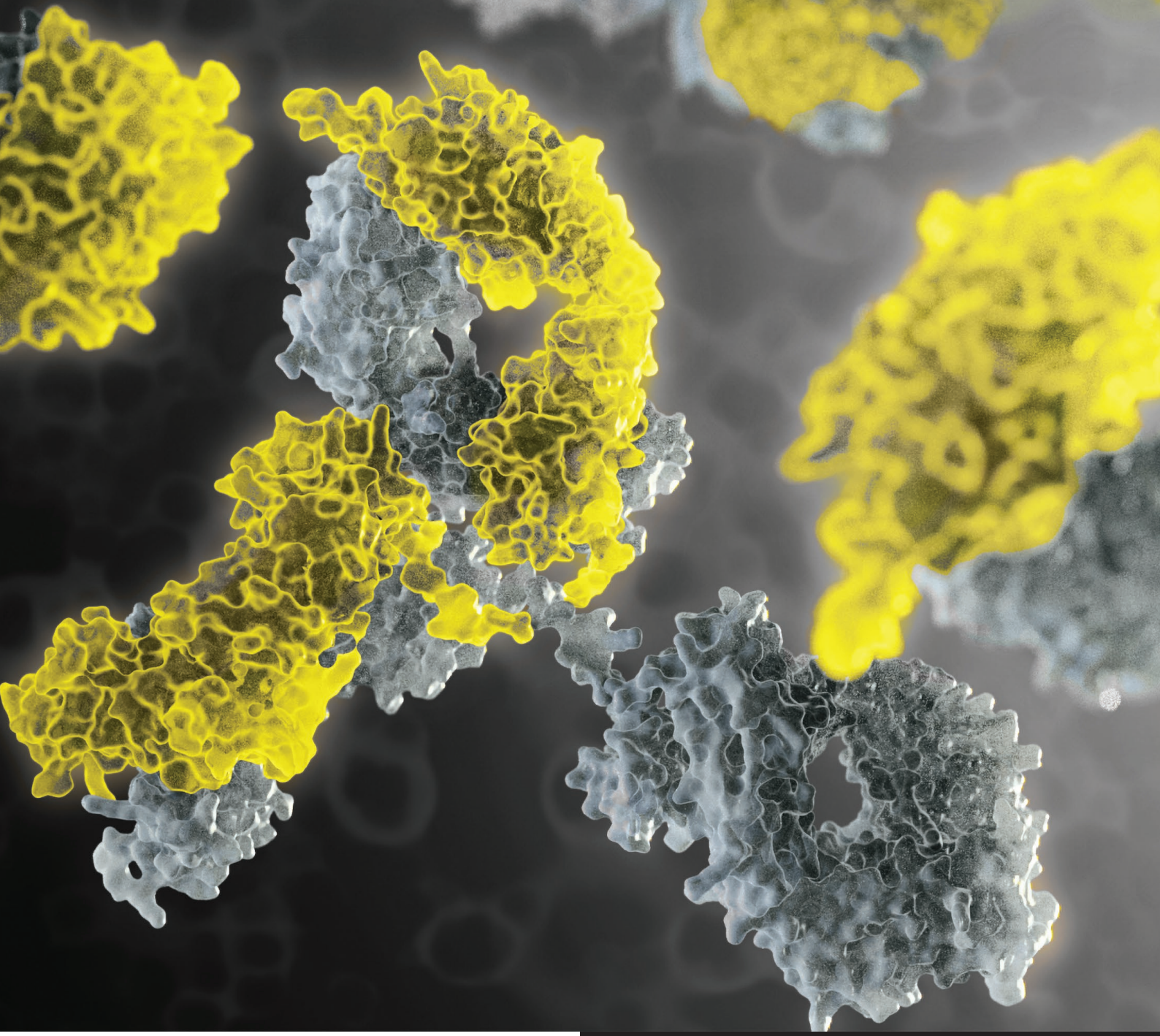


Fully Automated Image-Based Screening and Isolation of Clones and Single Cells for Antibody Discovery

CellCelector Flex

Simplifying Progress

SARTORIUS

CellCelector Flex: A Useful Tool in Antibody Discovery and Production

The identification and selection of optimal clones that secrete high levels of the desired antibody is one of the critical and time-consuming steps in the antibody discovery process. Using the CellCelector Flex, a fully automated image-based screening and isolation platform, significantly accelerates the process compared to conventional techniques.

Automation
- Automated screening, ranking, selection, and isolation process

Speed
- One-day B-cell screening workflow
- High-throughput single cell cloning (HT-NIC)

Flexibility
- B cells, hybridoma, Chinese Hamster Ovary (CHO) single cells, and clones
- Isolation from liquid and semi-solid media

Characterization
- Monoclonality
- Viability
- Functionality
- Productivity

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)

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

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

Find out more
www.sartorius.com

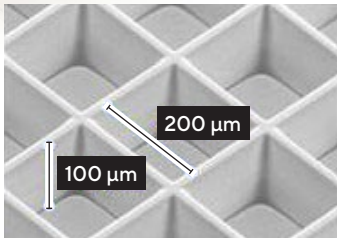
High-Throughput Identification of Monoclonal High Producer Clones

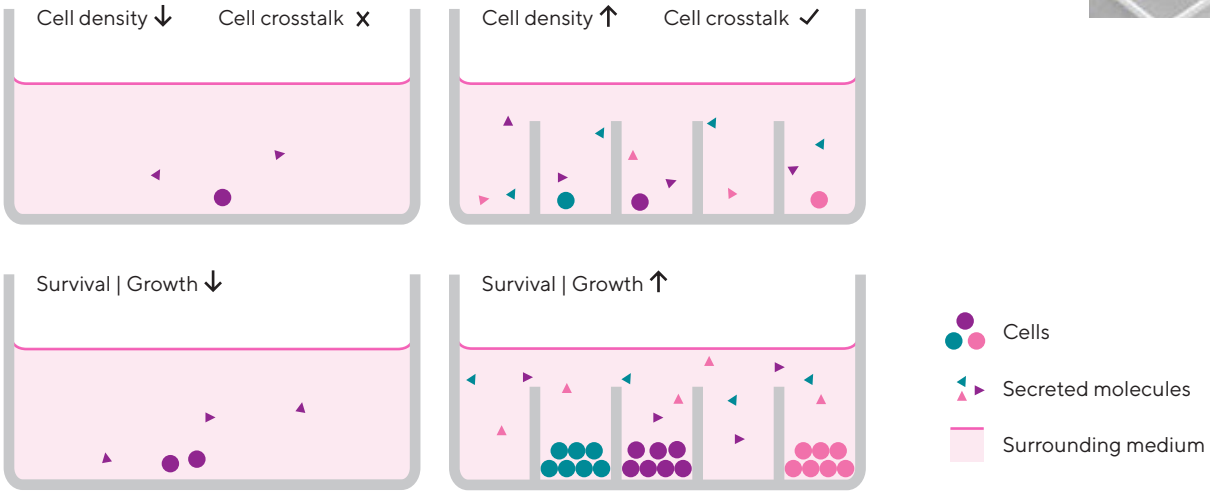
The nanowell-based image-verified cloning method (HT-NIC: High-Throughput Nanowell-based Image-verified Single Cell Cloning), a new technique developed for the identification of monoclonal high-producer clones combines the CellCelector Flex with our nanowell plates. With this new technique it is possible to effectively assess and verify the clones before deciding which ones to choose. In less than one week, your pool of single cells develops into monoclonal, viable, and productive colonies. 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 valuable time by avoiding missteps, a second cloning round, and unnecessary procedures.

Contrary to a traditional methylcellulose-based approach the CellCelector nanowell-based method allows the colony growth in liquid media. Clones are sharing the same media, but are effectively separated from each other by the nanowell walls.

CellCelector Nanowell Cell Culture Plates

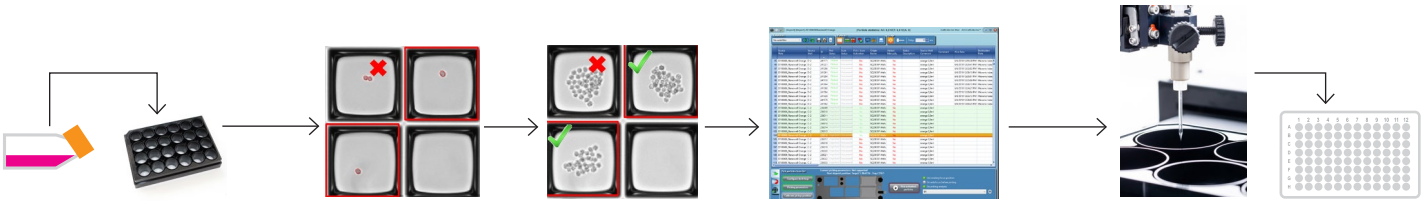
The HT-NIC method is based on the CellCelector nanowell plates. These plates are available in different formats featuring thousands of nanowells at the bottom of each well. For a 24-well plate this results in 4,300 nanowells per well or 100,000 nanowells per plate. In spite of the local separation, the cells in the nanowells are covered by the same medium and thereby allowing cellular crosstalk to 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.





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. Seeding multiple wells allows users to start with up to ~14,000 single cells captured in individual nanowells—all within just one plate.



Cell seeding into a nanowell plate

Monoclonality screening (Day 0)

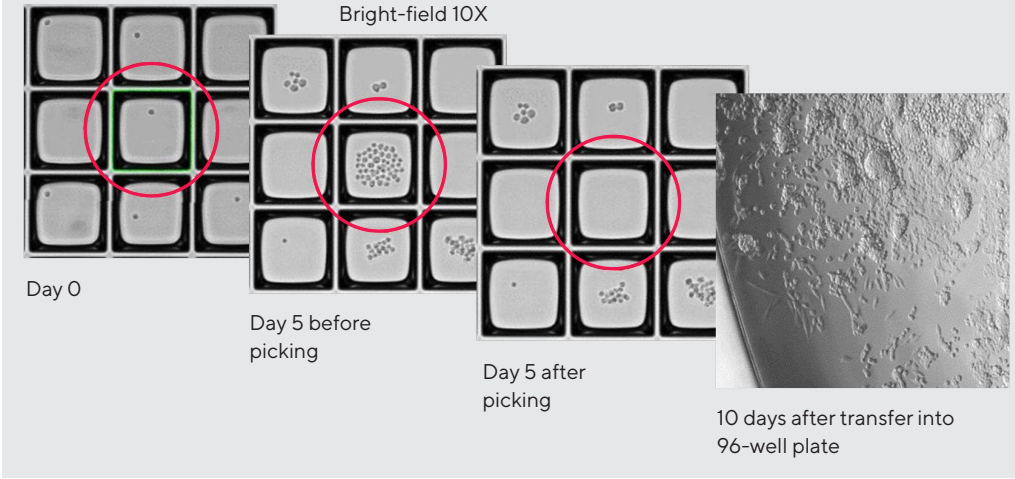
Clone growth assessment (Day X)

Automated clone ranking and selection

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

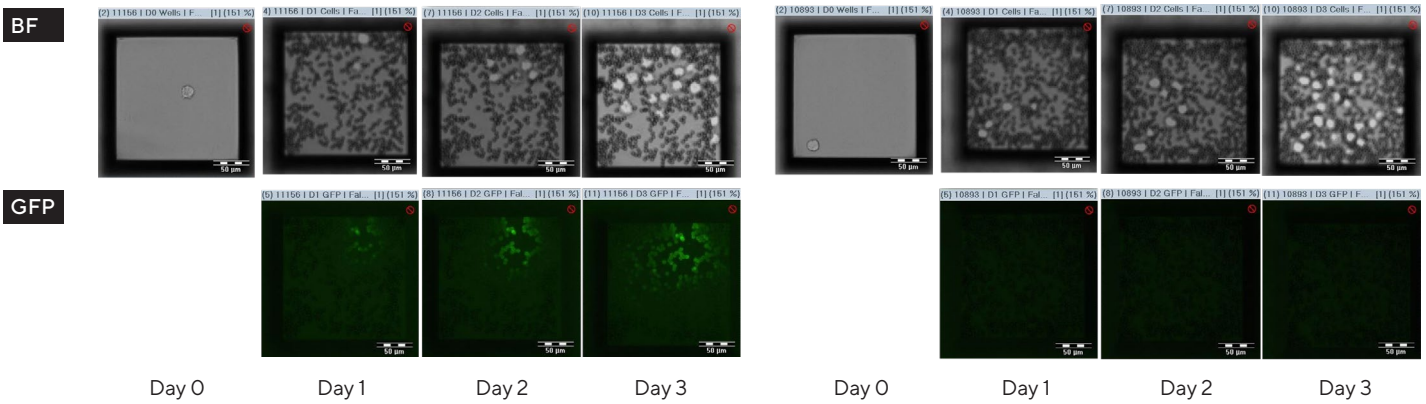
After monoclonality has been documented, cells are incubated for 3 to 6 days resulting in up to 75 cells per clone.

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.



Clonal Secretion Assays

In the nanowells, appropriate secretion assays can also be performed to ensure that only those clones which produce high levels of the desired antibodies are selected.



Highly secreting single cell clones. Images were taken in bright-field and fluorescence after 1, 2 and 3 days of cultivation. The bright-field image in Day 0 shows the single cell.

Single cell clones with no secretion of the desired antibody. Images were taken in bright-field and fluorescence after 1, 2 and 3 days of cultivation. The bright-field image in Day 0 shows the single cell.

Automated Single Plasma-B-Cell Secretion Screening and Recovery

Together with our unique nanowell technology the CellCelector Flex combines selective cell screening, imaging, sensitive single cell assays as well as cell isolation for parallel processing of thousands of single plasma B cells within one day.

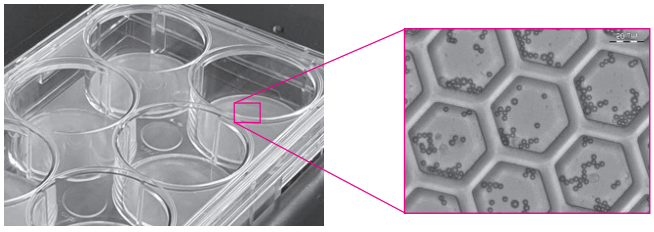
Single cell analysis also allows the detection of rare antibodies with unique properties which are hard to find under conventional screening methods. Individual cells will be immediately tested in several assays instead of culturing for weeks to reach a minimum number of cells for the assay.

CellCelector One-Day B-Cell Screening and Isolation Workflow

Cell Loading (10 min)	Single Cell Screening (15 min)	Secretion Assay (2–3 hr)	Cell Transfer (1 hr)	Report (15 min)
Hand cell seeding of single cell suspension in CellCelector nanowell array	Identification of single cell nanowells	Automated detection of secreting cells (hits)	Export of hits to PCR plates for downstream processing	Generation of the experiment documentation

Step 1. Easy Cell Seeding

The single cell suspension is dispensed manually into the wells of the nanowell array. Each well of the nanowell array is characterized by an additional well pattern on its bottom. Depending on the plate type as well as the size of the nanowells, this results in between 60,000 and 200,000 nanowells per well. After single cell solution is added, the individual cells are randomly distributed in the nanowells according to the Poisson distribution.



H 100 array: hexagonal 100 µm nanowells seeded with B cells and antigen-specific coated beads

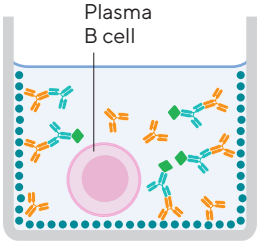
Step 2. Single Cell Screening

The seeded wells are scanned with the CellCelector Flex in bright-field. The software automatically identifies single cell nanowells.

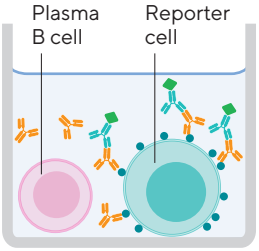
Step 3. Running the Single Cell Secretion Assay

The specific secretion assays are performed in the nanowells on the individual cells and allow the rapid identification of secreting cells of interest. The CellCelector B-cell workflow supports several types of cellular assays.

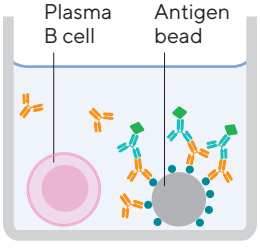
Example Assays



Assay with plate capture coating



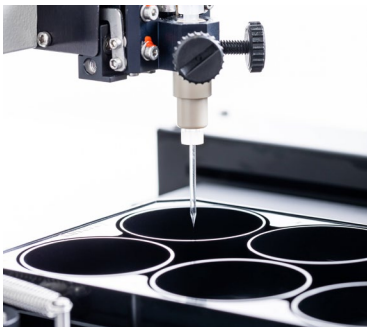
Antigen-expressing reporter cell assay



Bead-based assay

Step 4. Cell Transfer

After identification of the desired cells (hits), the CellCelector transfers them to the appropriate target vessels for subsequent analysis and processes.



The CellCelector facilitates a very gentle picking process based on mechanical aspiration, resulting in high rates of viability of the picked B cells.

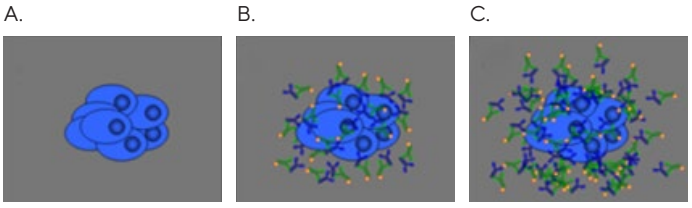
Step 5. Generation of the Report

The entire process from single cell screening to transfer of the hits is fully documented and compliant to GLP and GMP standards. High quality microscopic images of each picking process allow a later review. All imaging and numeric data (e.g. morphology and spectral values) as well as source and destination positions of each cell are stored in the database and can be exported quickly and easily into the customer's LIM system.

Selection of High-Producer Hybridoma and Chinese Hamster Ovary (CHO) Cells in Semi-solid Media

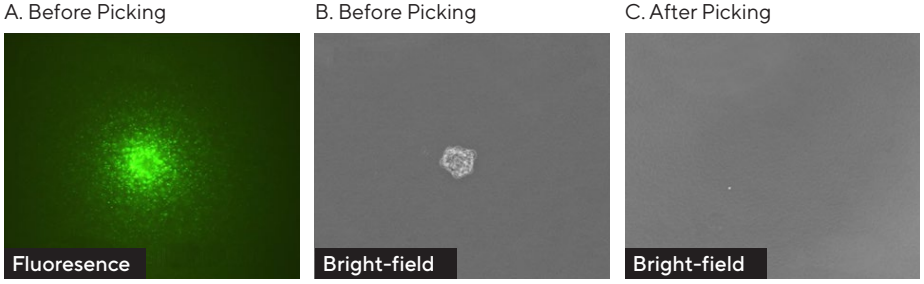
The antibody generation of hybridoma clones can vary dramatically between different clones. The proteins, produced by the clones and secreted into the medium, can be made visible by fluorescence-labeled secondary antibodies. As the viscosity of the semi-solid media prevents the antibody from diffusing further into the media, the fluorescence signal will appear as a halo around the cell clone that produced it. To specifically select highproducer clones, the CellCelector software compares the size of the cell colony (visible in bright-field) with the size of the fluorescent halo surrounding the cell colony and thus calculates the antibody production rate of each clone. This allows ranking of cell clones for best antibody production across multiple source plates.

The intensity of antibody production correlates with the diameter of the fluorescent halo around the colony: colony producing no antibodies (A and D), colony producing medium amounts of antibodies (B and E); colony producing high amounts of antibodies = high producer (C and F). The size of the colonies is similar (see enlarged area in bright-field observation D-F).



A-C: Schematic view of hybridoma clones (light blue) and the detection of secreted antibodies (dark blue) by fluorochrome labeled secondary antibodies (green with yellow circle).

D-F: Microscopic view (overlay of bright-field and fluorescence illumination) of hybridoma clones after incubation with fluorochrome labeled secondary antibodies.



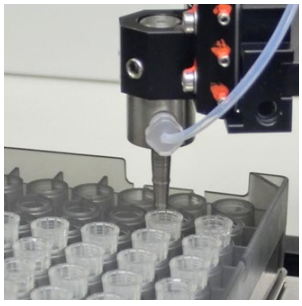
Antibody-producing CHO cell colony; images before and after picking with the semi-solid media module of the CellCelector Flex.

Precise Isolation of Hybridoma and CHO Colonies Out of Methyl Cellulose Containing Medium

For gentle and targeted isolation of clones from semi-solid medium, such as methylcellulose, agar, Matrigel®, etc., the CellCelector Flex offers a dedicated tool using specific disposable plastic tips. Depending on the dimension of the colonies to be picked two different diameters are available: 500 and 1200 µm.

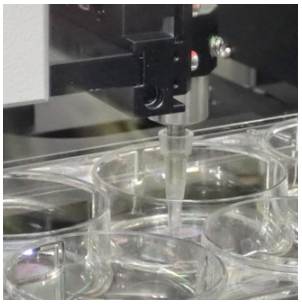
Workflow (20–30 s)

Tip Uptake



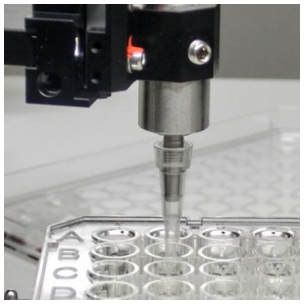
Taking of a new plastic tip from the rack

Clone Aspiration



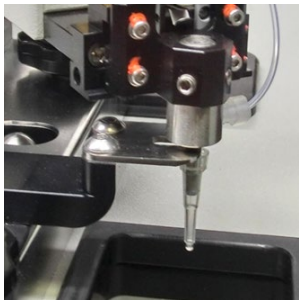
Picking of the target colony through controlled aspiration

Deposition



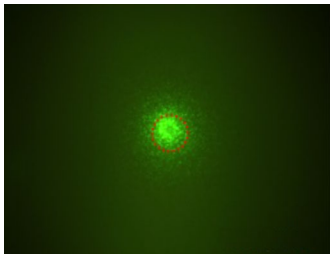
Delivery of the picked colony to the destination well

Tip Disposal

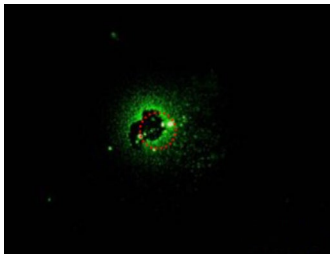


Disposal of the used tip to the bin

A.



B.

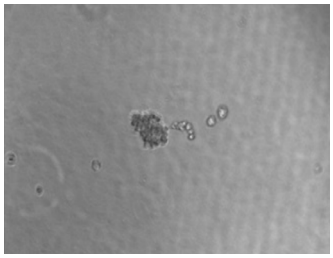


Fluorescence images of a CHO cell colony in methylcellulose before (A) and after (B) picking

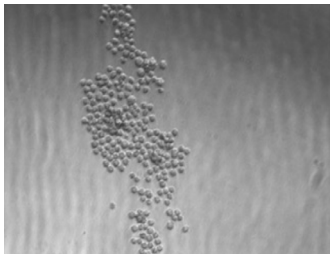
High Rate of Cell Survival After Transfer

Transfer of colonies is often stressful for the cells, resulting in a great number of dead cells influencing their living neighbors. Therefore, it is crucial to use a sensitive mechanical transfer method causing the least amount of dead cells. The CellCelector offers a gentle and reliable way to isolate and transfer Hybridoma colonies out of methyl cellulose containing media. The cells rapidly continue to grow in their new environment.

A. Day 1



B. Day 5



CHO cell colony harvested with the semi-solid media module cultured in a 96-well destination plate.

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Status: 06 | 2022