Miltenyi Biotec

In cooperation with:

SEED • Biosciences

MACSQuant® Tyto® Cell Sorter and DispenCell

Combining cell sorting and single-cell deposition

Background

Gentle sorting and single-cell deposition are crucial tools in cell line development, isolation of rare cells, CRISPR-based techniques, single-cell genomics, monoclonal antibody development, and many other applications. Single-cell analysis is helping to unravel population heterogeneity, identify populations of interest, and identify characteristics of individual cells. Functional cells with high quality are mandatory to get unbiased results in sensitive methods such as single-cell sequencing. In cell line development, it is crucial to reliably get single cells to grow monoclonal cell lines. Traditional methods such as limiting dilution require laborious hands-on work and are unfortunately not efficient. Single-cell deposition by conventional droplet sorters is a relatively harsh procedure that exposes the sorted cells to decompression, high shear forces, and charge. Cell sorting with the MACSQuant Tyto Cell Sorter is truly gentle.

The patented microchip technology enables cell sorting without decompression, or charge and only operates at low pressure (fig. 1). Additionally, the MACSQuant Tyto Cell Sorter requires minimal user training and is operated in a walk-away fashion without the need of monitoring droplet delay, for example. Similarly, the compact DispenCell allows a gentle single-cell deposition in a reliable and traceable manner. A single-cell analysis software tool provides a traceable proof of clonality report instantly.

The simple interface of the MACSQuant Tyto Cell Sorter allows easy adoption with minimal training. No cleaning or calibration is required resulting in a faster workflow. Thus, with the MACSQuant Tyto Cell Sorter and DispenCell, laborious applications involving single-cell isolation methods can be conducted in a more efficient manner.

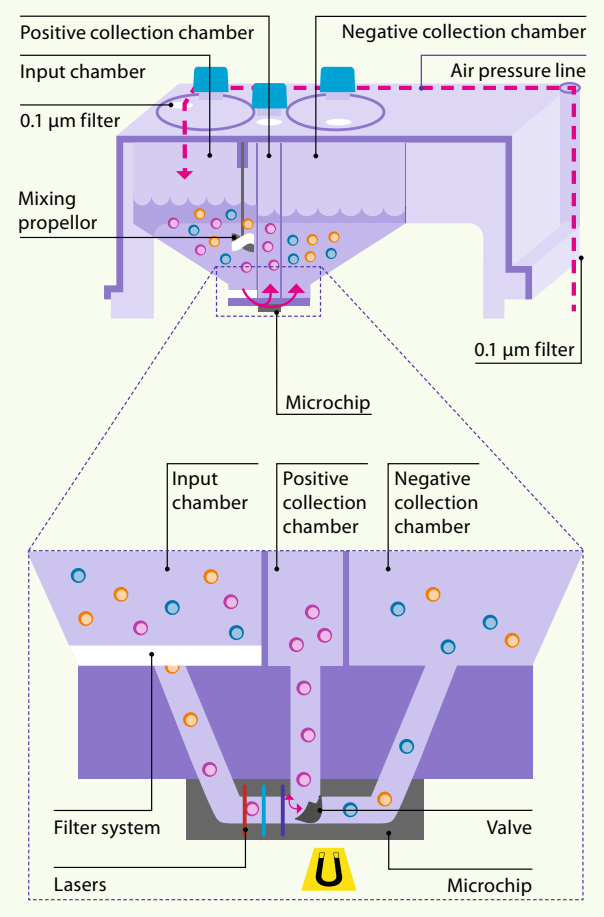

Figure 1: The sorting mechanism of the MACSQuant Tyto Cell Sorter. Cells leave the input chamber and cross a filter system before entering the microchip and crossing three lasers. When a target cell (pink) is identified, a magnetic pulse opens the microvalve, which redirects the target cell into the positive collection chamber. In the default state, the valve is closed, directing non-target cells (blue and orange) into the negative collection chamber.

The MACSQuant Tyto Cell Sorter can be easily combined with the DispenCell whose unique technology allows extremely gentle handling of the cell sample, comparable to manual pipetting, yet more efficient. Consequently, cell viability and outgrowth are preserved. The open deck contains two separate carriers to load 96- or 384-well plates. The dispensing head with its dispensing unit can move freely on a 3-axes robotic stage. A patented disposable tip ensures clean isolation of single cells and no cross-contamination. DispenCell's single-cell dispensing unit is fitted with a sensing tip that acts as a Coulter counter to detect the passage of the cells.

As each cell advances through the Coulter aperture to flow into the well, a unique electrical signal is triggered. The electrical trace that appears as a unique peak represents a single cell, whereas multiple peaks result from doublets or multiple cells (fig. 2), and a small amplitude peak is the mark of debris.

At the end of the experiment, single-cell analysis software provides a map for immediate and traceable proof of clonality. The full set of data is stored in the proof of clonality report.

Here, we use CHO cells, a cell line that is often applied in monoclonal antibody production. CHO cells are stained via CFSE and then spiked into unstained cells to reach a CFSE-positive cell frequency of 10%. Then, CFSE-positive cells are sorted on the MACSQuant Tyto Cell Sorter. DispenCell performs the single-cell deposition into 96-well plates. First, the single-cell deposition efficiency is assessed. In the end, a simulation shows the plating efficiency of different workflow scenarios.

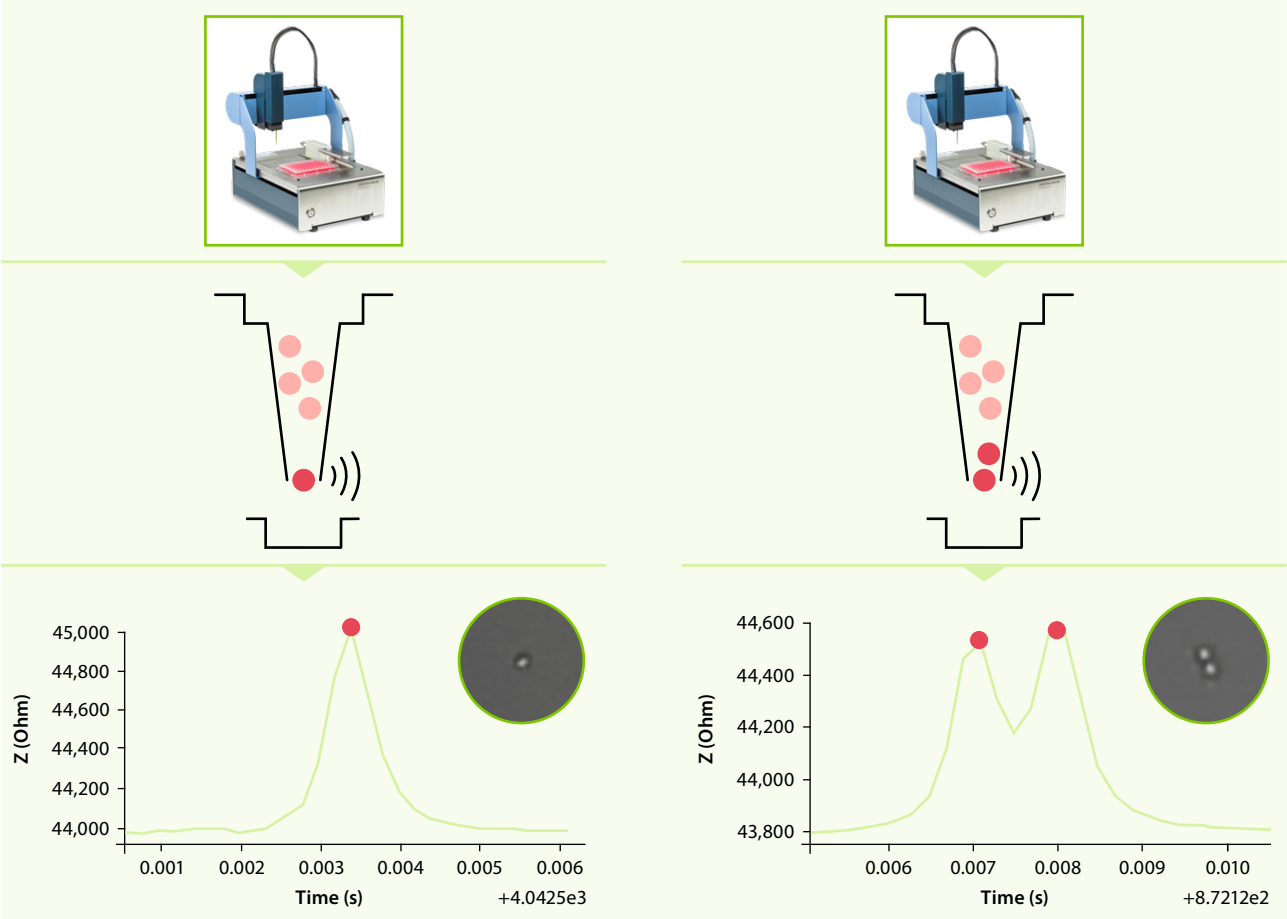

Figure 2: The cell deposition mechanism of the DispenCell. Single-cell deposition is based on impedance.

Methods

Model cell line and culture conditions
CHO K1 cell line, cultured under standard conditions (37 °C, 5% CO_2) in RPMI medium containing 10% FCS and pen/strep, was used for the experiment. Before the experiment, cells were split every 2–3 days at 1:5 or 1:10 dilution. On the day of the experiment, cells were resuspended using trypsin-EDTA (0.05%, phenol red). After cloning, 96-well plates (flat bottom) were incubated at the same conditions for another 6 days.

Cell preparation for sorting
Cells were stained with CFSE. After washing with RPMI 10% FSC medium, stained cells were mixed with the unstained cells in a 1 to 10 ratio to reach ~10% CFSE-positive cells in the mixture. Further, the cell mixture was stained with 7-AAD Staining solution (# 130-111-568) viability dye. After washing with PBS, cells were resuspended in 5 mL MACSQuant Tyto Running Buffer for sorting.

Cell sorting by MACSQuant Tyto Cell Sorter
For sorting, cells were resuspended in MACSQuant Tyto Running Buffer at a concentration of ~3.5×10^6 cells/mL. Cells were transferred into a primed MACSQuant Tyto Cartridge HS using a 10 mL syringe and a Pre-Separation Filter (20 µm) attached to the input chamber. Tyto Cartridge HS, the high-speed version of the MACSQuant Tyto Cartridges, facilitates a significantly reduced sorting time while increasing sort performance through inertial focusing. An aliquot of input fraction was taken for further analysis. Sorting was performed using software version 1.0.1, at 4 °C, and the sort gate was set on 7-AAD- $CFSE^+$ cells. After sorting, aliquots were taken from positive and negative collection chambers.

Manual cloning
Cells were diluted to a concentration of 1.25 cells/mL in the culture medium (RPMI, 10% FSC, pen/strep) and plated into flat-bottom 96-well plates, 200 µL per well, resulting in 0.25 cells/well. Plates were incubated under standard conditions (37 °C, 5% CO_2) for 6 days until the colonies formed in the wells were enumerated.

Cloning by DispenCell
Cells were counted on a MACSQuant Analyzer 10 and resuspended to a concentration of 2×10^5 cells/mL in MACSQuant Tyto Running Buffer. From this suspension, 50 µL of cells were transferred into 450 µL DispenME buffer to the final concentration of 2×10^4 cells/mL. Cells were deposited into a flat-bottom 96-well plate, one cell per well, with 200 µL RPMI, 10% FCS, pen/strep. Clone expansion (colony formation) was evaluated under a microscope 6 days after seeding.

At the end of the cloning process, DispenCell provides a report, indicating the wells that were deposited with one cell and the ones potentially containing more than one cell.
For cloning efficiency evaluation only the wells reported to contain one cell were verified for the presence of the colony. The wells reported to contain more than one cell, were treated as failed in terms of cloning.

Cloning efficiency evaluation
Six days after seeding, plates were evaluated under the microscope for the presence of cell expansion. Successful clone formation was defined by one colony per well, consisting of more than 20 cells.

The following three experimental conditions have been compared:

1. Target cell sorting using MACSQuant Tyto Cell Sorter followed by manual cloning
2. Target cell sorting using MACSQuant Tyto Cell Sorter followed by cloning by the DispenCell
3. Freshly cultured non-enriched cells, cloned manually

Results

Sorting of $CFSE^+$ CHO K1 cells
A mixture of cells with ~10% $CFSE^+$ cells was sorted on MACSQuant Tyto Cell Sorter using the gating hierarchy schematically depicted in figure 3. Target cell frequency was enriched from 9% in the input fraction to 98% in the positive fraction (fig. 4). In addition, 94% of target cells were depleted from the input fraction, as demonstrated by 0.49% target cells frequency in the negative fraction.

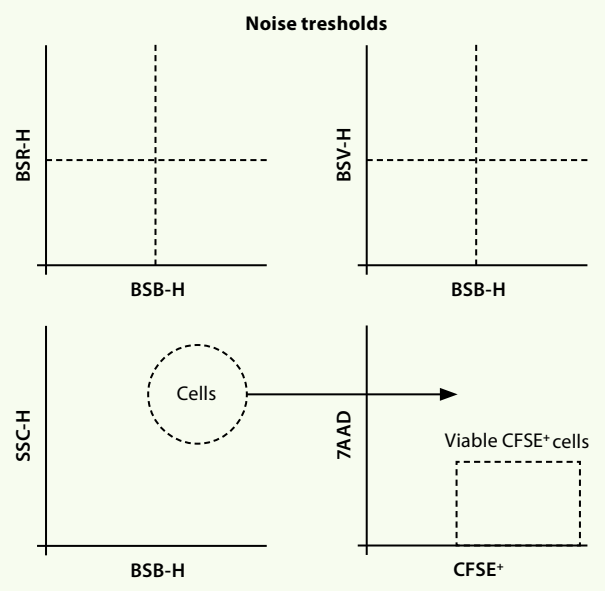

Figure 3: Gating strategy on MACSQuant Tyto Cell Sorter. Noise threshold is based on the backscatter signals of the cells. Cells falling above the threshold are used for further gating. The sort gate is defined as 7-AAD-$CFSE^+$ cells

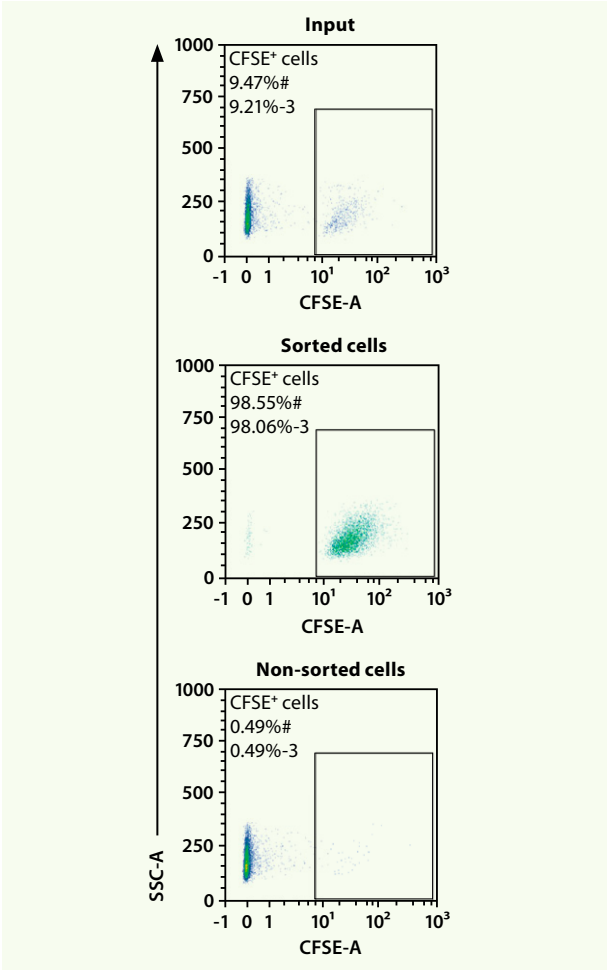

Figure 4: CFSE⁺ cells sorted using MACSQuant Tyto Cell Sorter. Results of sort demonstrate the frequency of target cells in input chamber, positive and negative collection chambers.

Manual cloning of sorted CFSE⁺ cells

Sorted CFSE⁺ cells were manually cloned into 96-well plates with the intention to plate 0.25 cells per well, which would result in 19% of the wells with a single cell and 2% with two cells, according to Poisson statistics. On day 6 of culture, 28 out of 192 wells (15%) presented with one colony (fig. 5), and 1 out of 192 wells (0.5%) with two colonies.

Cloning of sorted CFSE⁺ cells using DispenCell

Sorted CFSE⁺ cells were cloned into 96-well plates (one cell per well) using DispenCell. After counting the colonies after 6 days in culture the following results were obtained: 100 out of 190 wells (52%) contained one colony (fig. 5), and 5 out of 190 wells (2.6%) contained two colonies. Representative images of colonies from 0, 3, and 6 days of culture are shown in figure 6.

As an additional control, freshly cultured cells were cloned into 96-well plates (1 cell per well) using DispenCell. 6 days after cloning, 94 out of 191 wells (49%) presented with a single colony, and 6 out of 191 wells (3%) with two colonies (fig. 5).

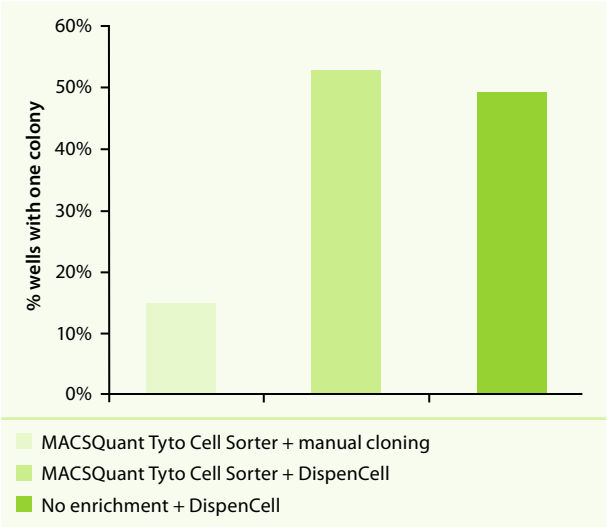

Figure 5: Cloning efficiency at different experimental conditions. Percentage of wells in a 96-well plate containing one colony on day 6 after cloning at three experimental conditions: **I)** cells sorted on the MACSQuant Tyto Cell Sorter and manual cloning, **II)** cells sorted on the MACSQuant Tyto Cell Sorter and cloning with DispenCell, and, **III)** freshly cultured, non-enriched and cloned cells with DispenCell. At least 190 wells from two 96-well plates have been analyzed for every experimental condition.

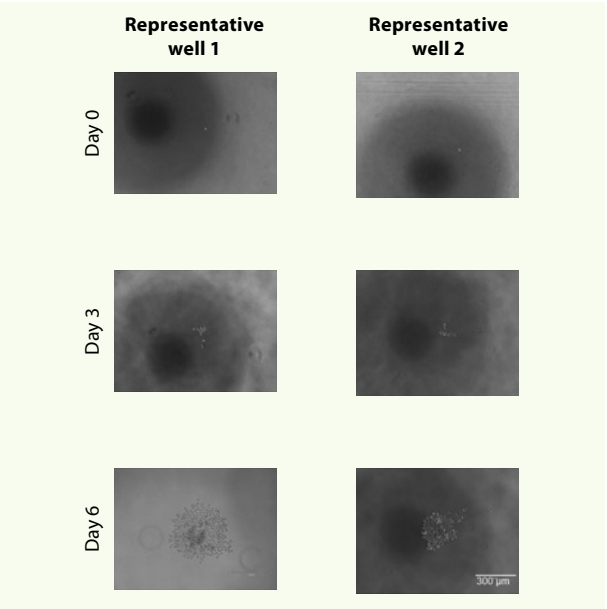

Figure 6: Microscopic images of cells in a 96-well plate. Images were taken using a 10× objective on day 0, day 3, and day 6 after cloning. Two representative wells are shown. The white bar represents 300 µm.

Evaluation of the efficiency of different workflows
Based on the combination of experimental data and Poisson statistics we compared four hypothetical experimental conditions (fig. 7):

A. Cell mixture containing 10% target cells is manually cloned into 96-well plates at a frequency of 0.25 cells/well. According to Poisson statistics, 19% of wells would contain one cell under this condition. 10% of these wells would contain target cells. Altogether, this would mean that 1.8 wells out of 96 wells (1.9%) would contain a target cell (96 × 10% × 19%). This hypothetical condition represents an experimental setup in which no target cell enrichment is performed and cell cloning is performed manually.

B. Cell mixture containing 10% target cells is cloned using DispenCell into 96-well plates at a frequency of one cell/well. According to our experimental data, 49% of wells presented with one colony 6 days after cloning. This indicates that at least 49% of the wells contained a single cell as a result of the cloning procedure. For this simulation, in order to simplify calculations, we assume that every cell cloned into a well gave rise to a colony. With a target cell frequency of 10%, we can expect that 4.7 wells out of 96 (4.9%) will contain a target cell (96 × 10% × 49%). This hypothetical condition represents an experimental setup in which no target cell enrichment is performed and cell cloning is performed using DispenCell.

C. Target cells enriched to 98% purity are manually cloned into 96-well plates at a frequency of 0.25 cells/well. Based on Poisson statistics and target cell frequency, 18 wells out of 96 (18.8%) would present with a target cell (96 × 98% × 19%). This hypothetical condition represents an experimental setup in which target cells were sorted to 98% purity using the MACSQuant Tyto Cell Sorter and were further manually cloned.

D. Target cells enriched to 98% purity are cloned using DispenCell into 96-well plates at a frequency of one cell/well. According to our experimental data, 52.6% of the wells contained a single colony on day 6 after cloning. Same as in condition **B,** we assume that every cell cloned into a well gave rise to a colony. With a target cell frequency of 98%, we can expect that 49.5 wells out of 96 (51.6%) will contain a target cell (96 × 98% × 52.6%). This hypothetical condition represents an experimental setup in which target cells were sorted using the MACSQuant Tyto Cell Sorter to 98% purity and were further cloned using DispenCell.

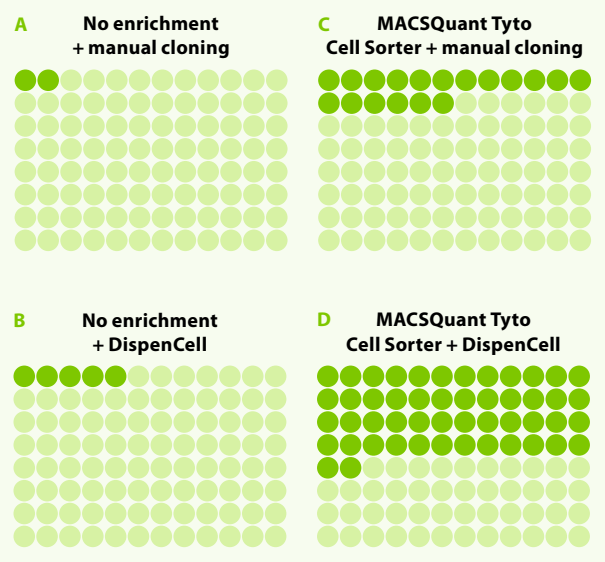

Figure 7: Workflow efficiency estimation

Conclusion

In this study, CHO cells were used as a model to demonstrate the power of combining the MACSQuant Tyto Cell Sorter and DispenCell for gentle cell sorting and single-cell deposition.

After enriching target cells with MACSQuant Tyto Cell Sorter, 49% cloning efficiency was reached when DispenCell was used for single-cell deposition. At the same time, only a 15% cloning efficiency was observed when manual cloning was used instead of the DispenCell device. Additionally, based on our model, only 1.9% cloning efficiency would be reached if unsorted (non-enriched) cells would be manually cloned. Therefore, the workflow involving both instruments increases the probability of successful target cell cloning by enriching the cells of interest via sort and by replacing the highly inefficient manual cloning with an automated system.

Additionally, both the MACSQuant Tyto Cell Sorter and DispenCell require only minimal user training, and operate in a walk-away fashion. This enables non-experienced users to reliably execute such complex and sensitive procedures as cell sorting and cloning.

Product table	Order no.
Flow sorting*	
MACSQuant Tyto Cell Sorter	130-103-931
MACSQuant Tyto Cartridge HS	130-121-549
MACSQuant Tyto Running buffer	130-107-206
Flow cytometry*	
MACSQuant Analyzer 10	130-096-343
Cell cloning**	
DispenCell S2021	
DispenKit	
DispenMe	

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