Single-cell dispensing and screening of hiPSCs for monoclonality verification using the impedance-based single-cell dispenser and high-throughput fluorescence-based imager

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Summary

Previously we demonstrated a method of fast and efficient isolation of single cells using CHO and HEK cells while preserving their integrity and providing evidence for their clonality. However, compared to CHO and HEK cells, it is more difficult to obtain colonies from single human induced pluripotent cells (hiPSCs). In this app note, we show the impedance-based single-cell dispenser and automated fluorescence-based high-throughput imaging screening to obtain monoclonal cell lines of human pluripotent stem cells through robust single-cell selection.

Benefits

- Improve the efficiency and yield of the monoclonal cell-line development process.
- Increase single-cell dispensing throughput and reliability.
- Facilitate the development of several bioprocess developments and drug screening studies.

Methods

The workflow (Figure 1, steps 3 and 4) for single-cell dispensing, characterization of colonies, and monoclonality assessment includes an impedance-based single-cell dispenser (DispenCell™ Single-Cell Dispenser), and an automated cell imager (CloneSelect® Imager FL). Using this bundle, human iPSCs were dispensed into 96- and 384-well plates imaged on Day 0 for double monoclonality assurance (impedance and microscopy), and later monitored regularly for growth using CloneSelect Imager FL (CSI FL). The DispenCell's analysis software identifies single cells and introduces optimization parameters such as time between two events and time per plate to maximize the efficiency of single-cell dispensing. The cells were isolated and imaged using the following steps.

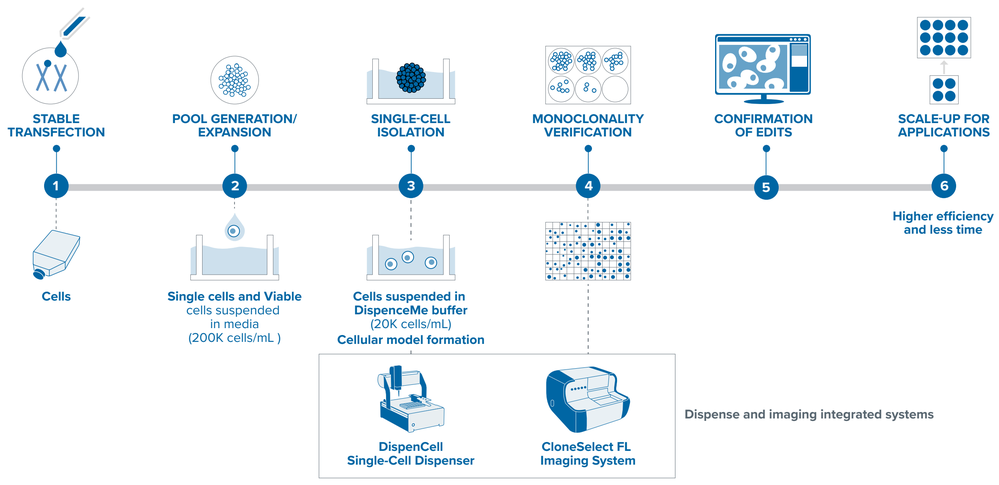


Figure 1. Cell line development workflow using DispenCell™ and CloneSelect® Imager FL (CSI-FL).

Cell culture

Human iPSCs (ATCC ACS-1023™) were cultured in Vitronectin XF ECM coated 6-well plates (Stemcell 07180) with mTeSR Plus Media, which contains Basal Media (Stemcell 100-274) supplemented with mTeSR Plus 5X supplement (Stemcell 100-275). Cells were passaged every 3 or 4 days using ReLeSR passing reagent (Stemcell 05872).

Experimental setup

Prior to any cell sample preparation, we prepared the mTeSR Plus Media supplied with Thiazovivin ROCK inhibitor (Selleckchem S1459). We then added 200µL of this media into each well of the 96-well plate and 50µL into the 384-well plate. The plates were incubated at 37°C with 5% CO_2 until use. The instrument was set up in a sterile cell culture hood and linked to the computer as per manufacturer's instructions.

Cell sample preparation

6-well plates with human pluripotent stem cells were treated with Accutase for five minutes at 37°C in an incubator. The cells were then diluted with cell culture media and filtered through the 20µm cell strainer (SEED Biosciences). Cell imaging on the countess cell counter and hemocytometer was performed to get accurate counts of cell concentration per mL and viability. The viability was preferred to be above 90% and the cells needed to be in a single-cell suspension. The cell concentration was adjusted to 200,000 cells/mL, concentration greater than 200,000 cells/mL would cause the time between cells during dispensing to be lower than three seconds, which could lead to having more than one cell in a well. On the contrary, concentration less than 200,000 cells/mL would increase the total dispensing time and therefore prolong the whole process, which may result in lower viability of the iPSCs.

Mix with DispenMe buffer

DispenMe buffer was provided by the kit and should be kept at 4°C before use. Gently pipetting up and down by using a wide bore tip to mix 15µL of the above-mentioned single-cell suspension with 135µL of DispenMe buffer without any delays (to prevent cell clumping). Avoid bubbles during mixing.

Cell culture

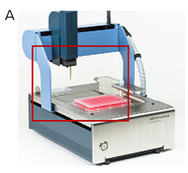
Follow the steps on the DispenCell manual for sample loading, plate settings, and device setup prior to dispensing (Figure 2).

A cell size threshold of 200 Ohm was selected (based on the particle size histogram generated during the sample loading).

Time between cells (Tcc) recommended range: ~ 2–5 seconds

Process (plate dispense) time ∝ Tcc ∝ Process Efficiency

A



B

C

Number of multiwell plates	Recommended loading time
1x 96 multiwell plates	30 sec
2x 96 multiwell plates	60 sec
1x 384 multiwell plates	120 sec

Figure 2. A. Image of a 96-well plate setup on dispenser holder being dispensed into. B. Plate 1 selected with a 96-well plate as an example. C. Recommended loading times for different plate types.

Methods

CSI-FL imaging

The dispensed plates were carefully moved to the incubator and imaged later using the CSI FL on Day 0 for Day 0 images, on Day 3 and Day 7 for colony outgrowth and finally on Day 10 for colony counts. Day 0 images were used for verification of monoclonality by CSI FL ality report feature.

Results

We demonstrated the feasibility of workflow using DispenCell and CSI FL as a bundle to dispense human iPSCs in 96-well plates. DispenCell has a unique technology which allows for extremely gentle handling of the cell sample. Cells are exposed to no more pressure than with manual pipetting (less than 0.1 psi). This allows the instrument to preserve cell viability and outgrowth while simultaneously increasing cell deposition efficiency. In addition, we confirmed monoclonality and monitored colony outgrowth by image-based data from CSI FL.

Automatic colony count

The Plate Thumbnails feature shows the entire plate and the wells that have been imaged (Figure 3). The thumbnails include the confluence overlay of each of the imaged wells. We used this screen to select wells to be included or excluded from analysis, which helps to focus only on wells of interest. Day 10 images were processed using Loci Count feature to count the number and measure the size (area) of colonies in each well (Figure 4A). We adjusted the upper and lower thresholds of the criteria to properly identify colonies. The criteria include the area (the colony's size) and the compactness (the colony's shape) (Figure 4B). The Loci Count feature of CSI FL automatically displays the number of colonies on each well (Figure 5).

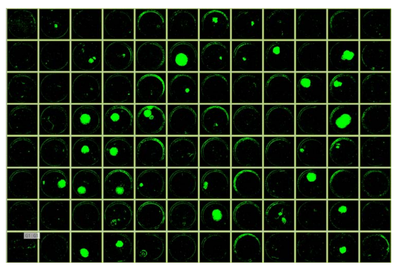


Figure 3. Whole well images on Day 10 generated by the CSI FL, showing colonies in green.

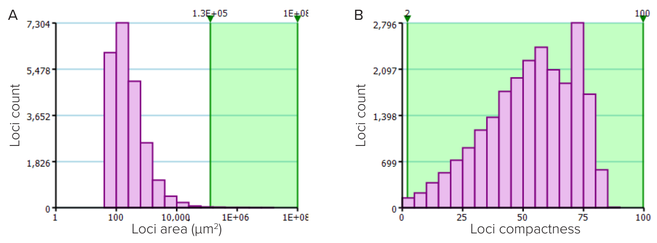


Figure 4. A. The Loci count feature showing the area of colonies in each well. B. the Loci count feature showing the compactness of the colony.

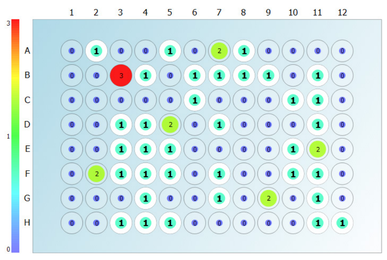


Figure 5. The Loci Count feature of the CloneSelect Imager FL displays the number of colonies in each well.

Colony outgrowth and screening

CSI FL software allows you to quickly toggle back and forth among the images at different time points (Figure 6). We identified the colonies using Day 10 images and examined whether the colonies were monoclonal or multi-clonal using Day 0 images. Figure 6 shows examples from four wells where the colonies come from single cells or multiple cells.

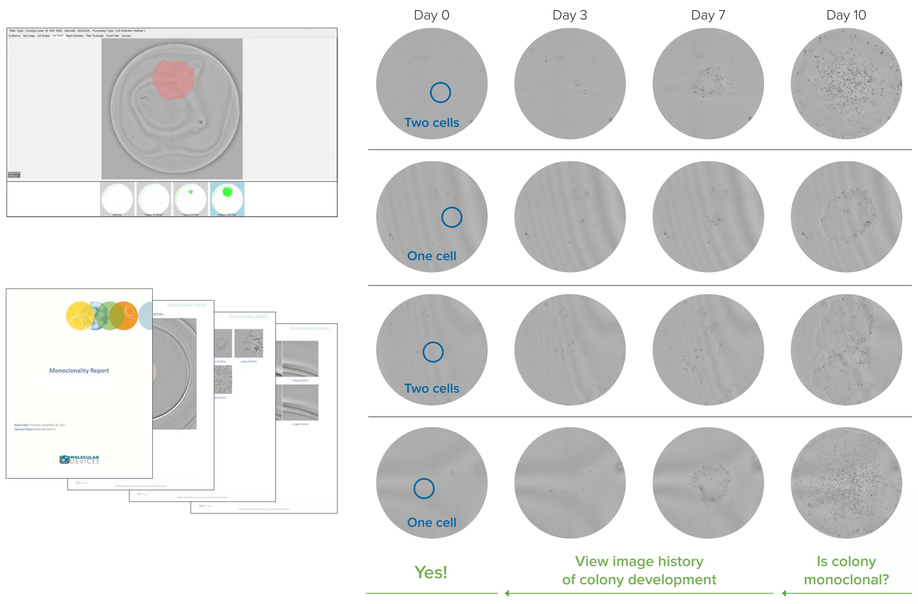


Figure 6. The CloneSelect Imager FL software allows you to toggle between images at different time points. This is an example of how the instrument helps differentiate and assure (Day 0 assurance) the presence of a cell versus multiple cells or debri on Day 0.

Monoclonality report

The Monoclonality Report feature of CSI FL provides image evidence of monoclonality in the cell line development process. With a few simple clicks, the Monoclonality Report feature on the CSI FL objectively organizes the supporting image evidence needed to establish clonality into an easily shareable report, saving researchers hours when compared to manual processes.

Conclusions and future directions

- This combination of the DispenCell Single-Cell Dispenser and CloneSelect Imager FL workflow provides an innovative and simple impedance-based method for the isolation of intact single cells and imaging.
- The DispenCell provides high efficiency and high viability for human iPSCs.
- The DispenCell and the CSI/CSI FL can be integrated into an automated workflow using a liquid handler, an automated incubator, and a collaborative robot.
- Its incorporation into an automated platform can increase in dispensing throughput and reliability and has the potential to facilitate gene editing.