TECHNICAL NOTE

SIMPLIFY CELL LINE DEVELOPMENT.

Combining Uno Single Cell Dispenser™ and Spark® Cyto multimode imaging reader to accelerate cell line development.

TECAN

INTRODUCTION.

Single cell cloning is a critical step in the development of monoclonal cell lines, with applications spanning therapeutic protein production, drug screening, gene therapies, and exploring cell function and disease mechanisms. Ensuring colonies originate from a single cell is essential to avoid discrepancies in product quality and recombinant protein instability, as well as to improve experimental reproducibility. The significance of clonal cells lies in their ability to contribute to reliable and standardized outcomes across diverse experimental conditions.

Limiting dilution is a common method for single cell cloning. It involves diluting cells down to low concentrations to obtain a single cell per well based on a Poisson distribution. However, limiting dilution is inherently inefficient, and most wells end up with no cells or more than one cell.[1] The most widely used method to isolate single cells is fluorescence-activated cell sorting (FACS), but commercially available instruments are complex, expensive and can impact cell health.[2]

This technical note describes the use of the Uno Single Cell Dispenser to isolate single cells. This instrument harnesses microfluidic digital dispensing technology to gently isolate viable cells for various downstream applications, including cell line development. The viability of the single cell post dispensing was assessed by addition of a fluorescent dye, that bound to the DNA of cells with impaired membrane integrity, using whole-well fluorescence imaging functions of the Spark Cyto. Cell outgrowth rate was determined 10 days after single cell isolation with the Uno, using whole-well brightfield imaging (Figure 1). Using Uno and Spark Cyto together, enables simple single cell isolation and determination of monoclonal cell outgrowth for variety of downstream application.

MATERIALS AND METHODS.

Mammalian cell culture: HeLa cells (DSMZ, ACC 57) were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, #41966-029), supplemented with 10 % fetal bovine serum (FBS, Gibco, #10270-098), 100 IU/ml penicillin and 100 µg/ml streptomycin (Sigma-Aldrich, # P0781) at 37 °C and 5 % CO_2 in a cell incubator.

Single cell isolation: Individual HeLa cells were isolated using the Uno Single Cell Dispenser with a C1a Uno Dispensehead Cassette™ (#30230841). A dispense protocol was created with UnoControl software, using a fluid class that corresponded to a medium cell size (15-17 µm), and applying this fluid to a 96-well plate to dispense one cell per well (Figure 2).

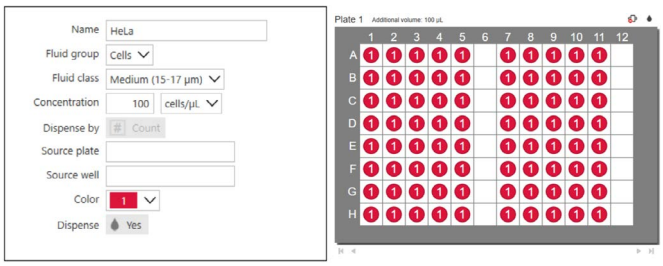

Figure 2: UnoControl software user interface. Left: a medium cell fluid class (15-17 µm) was selected to isolate HeLa cells. Right: the HeLa cell fluid was applied to a 96-well plate.

A cell suspension of approximately 100 cells/µl was resuspended in PBS without Mg^{2+} and Ca^{2+} (Gibco, #10010-015).[1] 20 µl of the suspension was added to the C1a Dispensehead Cassette, then cells were dispensed into 80 wells containing 200 µl DMEM supplemented with 10 % FBS and antibiotics. For cell viability assessment, the media of 40 wells were supplemented with CellTox™ Green Dye (Promega, #G8741), diluted 1:500. For the negative control culture medium was supplemented with CellTox Green Dye (background), for the positive control 10'000 cells were seeded, lysed and stained with CellTox Green Dye (as recommended by the manufacturer).

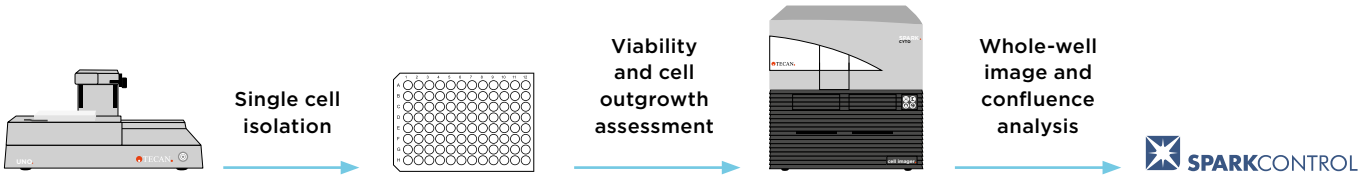

Figure 1: Cell line development workflow using Uno Single Cell Dispenser and Spark Cyto. Single HeLa cells were isolated into a 96-well plate using the Uno Single Cell Dispenser. Cell viability 1 h post dispensing was determined by fluorescence imaging (CellTox™ Green Dye) and cell outgrowth was analyzed by using Spark Cyto's confluence mask 10 days post dispensing.

Cell viability and single cell outgrowth analysis: The analysis of individual cell clones and cell viability assessment were performed using a Spark Cyto 600 multimode imaging plate reader (Tecan). Environmental conditions were optimized for the cells during the imaging process, with the temperature set to 37 °C and the CO_2 level adjusted to 5 %. Images were acquired one hour, one day and 10 days after single cell dispensing, and captured with a 2x objective in brightfield and green fluorescence imaging modes. Single cell viability was assessed one hour after cell dispensing. Overlaying images from brightfield and green fluorescence imaging channels enabled the detection of dead cells that were fluorescent. Cell outgrowth was observed by analyzing the images on day 10 using the Spark Cyto's confluence algorithm. Single cell origin outgrowth was calculated by counting the number of wells that contained a single colony, divided by the total number of wells where a single cell was dispensed.

RESULTS.

Single cell isolation using the Uno had an isolation success rate of 97 % across 80 wells of a 96-well plate (Figure 3), with a total run time of 2:48 minutes.

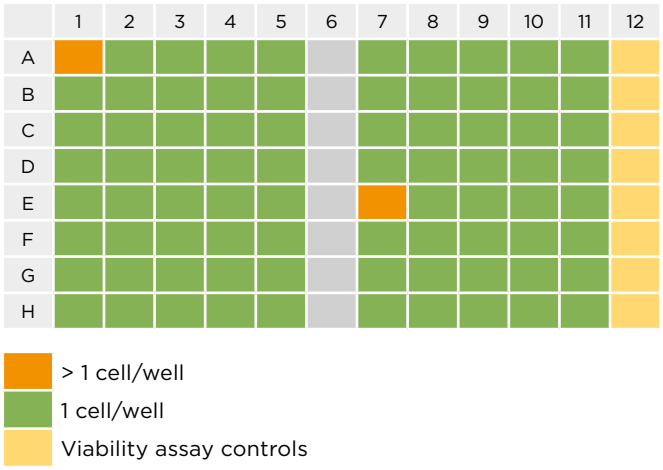

Figure 3: 97 % cell isolation success rate achieved with the Uno (day 0).

Following cell isolation, the wells were imaged using the Spark Cyto. The 2x objective was used to acquire whole-well images in a single field of view. This enabled quick and efficient preview of sample wells in both brightfield and fluorescence modes, optimizing the focal plane and imaging parameters. Cell viability one hour post dispensing was 90 % (4 wells out of 40 wells contained dead cells) (Figure 4).

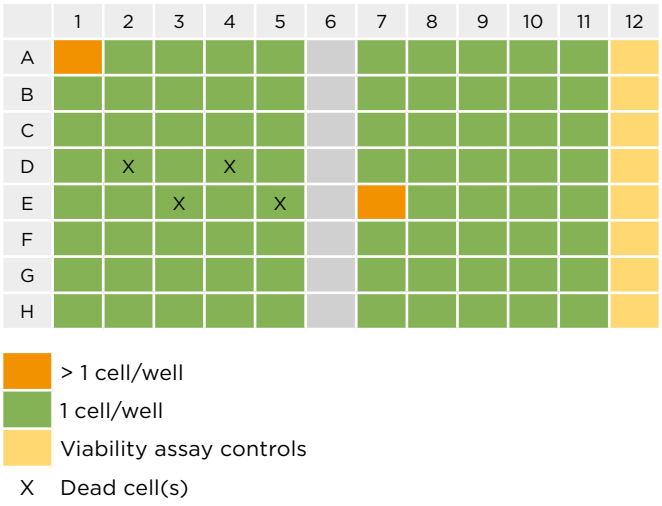

Figure 4: 90 % cell viability one hour post dispensing based on fluorescence imaging.

The plates were scanned on days 1 and 10 after single cell isolation to monitor clone growth over time (Figure 5). Images were analyzed using the confluence algorithm in Image Analyzer™ to identify the best-performing clones based on area coverage and phenotypic appearance. Overall, a 47 % cell outgrowth rate was determined.

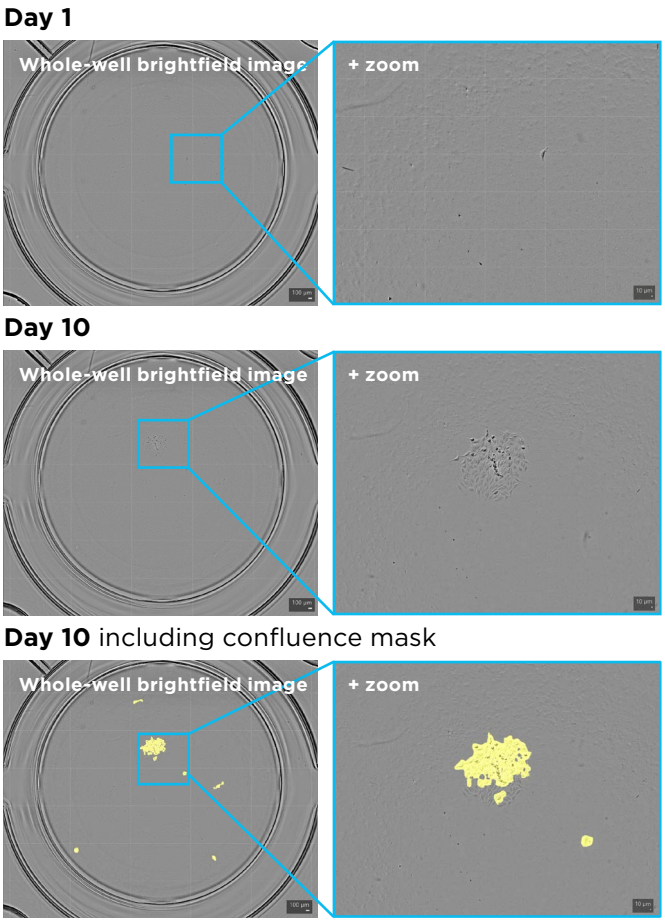

Figure 5: Colony detection with whole-well imaging. The Spark Cyto was used to acquire whole-well brightfield images in 96-well plates, which were automatically analyzed using Image Analyzer software to identify areas of cell growth (yellow mask).

CONCLUSION.

The method described here can be easily adapted for various research and drug development applications, helping to advance new therapies and our understanding of cellular processes. This technical note highlights the synergy between the Uno Single Cell Dispenser and Spark Cyto, showcasing their benefits in isolating cells, ensuring monoclonality and characterizing individual mammalian cell clones for cell line development.

REFERENCES.

1) Andre Gross *et al.* Technologies for Single-Cell Isolation. *Int. J. Mol. Sci.* 2015, 16(8), 16897-16919.
2) Elizabeth M. Llufrio *et al.* Sorting cells alters their redox state and cellular metabolome. Redox Biology, 2018, 16, 381-387.
3) Culture preparation for dispensing mammalian cells, Tecan Protocol

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