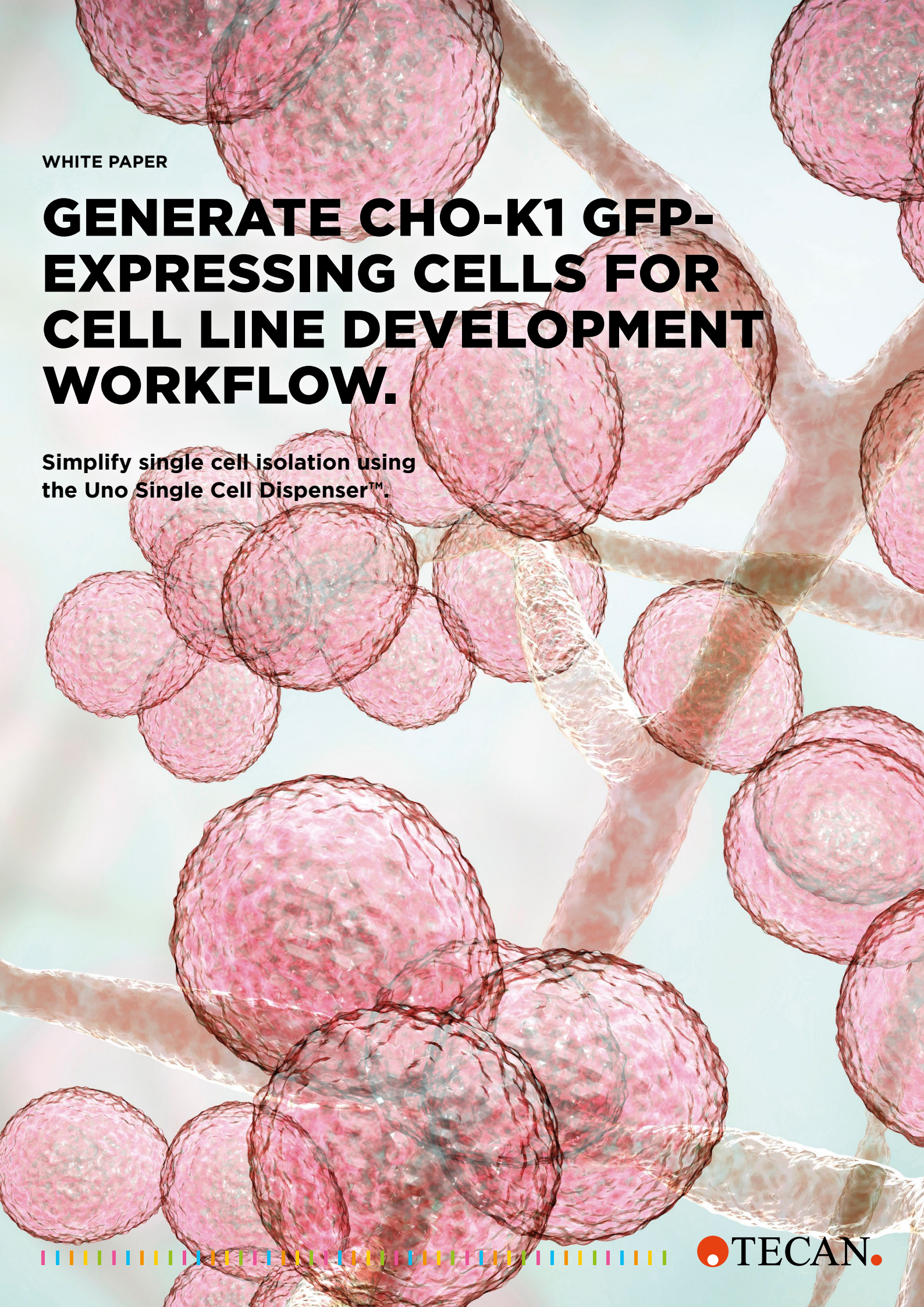

WHITE PAPER

GENERATE CHO-K1 GFP-EXPRESSING CELLS FOR CELL LINE DEVELOPMENT WORKFLOW.

Simplify single cell isolation using the Uno Single Cell Dispenser™.

TECAN.

INTRODUCTION.

Clonal isolation of single cells is a common method in the field of cell biology, and is used to generate cell lines carrying specific genetic mutations for numerous downstream cellular, molecular and biochemical applications. The resulting cell lines can be used to explore cellular signaling pathways, or simply to generate fluorescent-tagged cells for imaging or sorting functions. A single cell dispenser is a fast and efficient method to generate single cell clones with high fidelity and throughput.

Here, we use the Uno Single Cell Dispenser to isolate CHO-K1 cells previously transfected via electroporation with a gene construct to express green fluorescent proteins (GFP). We will also demonstrate the expansion of single clones into cell lines that stably express GFP, resulting in a CHO-K1 GFP-expressing cell line that can be used in the lab for single cell imaging workflows. Overall, these experiments showcase the versatility of the Uno Single Cell Dispenser for single cell isolation, supporting cell line development.

METHODS.

The Uno Single Cell Dispenser was used to establish a monoclonal CHO-K1 cell line that stably expressed GFP. A dispense protocol was prepared using a medium cell fluid class to isolate cells between 15 and 17 µm, which corresponded to the average size of the CHO-K1 cells used in the experiment. In this four-step procedure, we transfected CHO-K1 cells with a mammalian selection cassette (neomycin) containing a GFP expression vector, selected transfected cells with antibiotics, isolated single cells, and expanded GFP-expressing single cells into stable cell lines that expressed GFP.

Workflow for clonal isolation of GFP-expressing CHO-K1 cells.

CHO-K1 cells that did not previously express GFP were transfected with 2 µg/ml DNA using the Neon™ NxT Electroporation System and treated with G418 antibiotics for three weeks, before single cells were isolated and expanded into colonies for two weeks (Figure 1). Cells were initially treated with two doses of G418 (2 mg/ml) at 48 and 96 hours after transfection, followed by maintenance doses (0.2 mg/ml) in the culture media.

Single cell dispensing protocol.

The cell fluid class corresponding to a medium (15-17 µm) cell size was used to isolate CHO-K1 cells (Figure 2). The CHO-K1 cell fluid was applied to a 384-well plate by creating a dispense protocol to dispense one cell per well (Figure 3). Cells were isolated by running the protocol with the antibiotic-selected CHO-K1 cells.

Materials.

C1a Single Cell Dispensehead Cassette and CHO-K1 cell suspension.

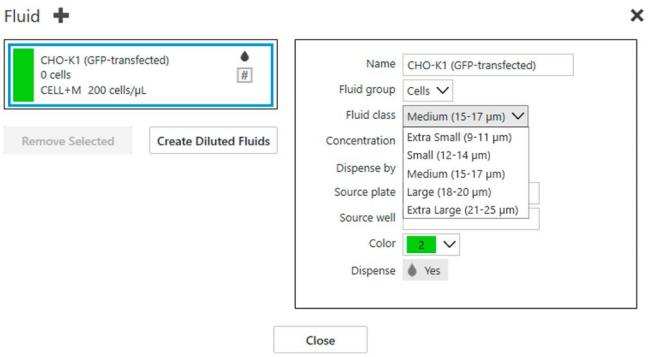

Figure 2: Medium cell fluid class selection.

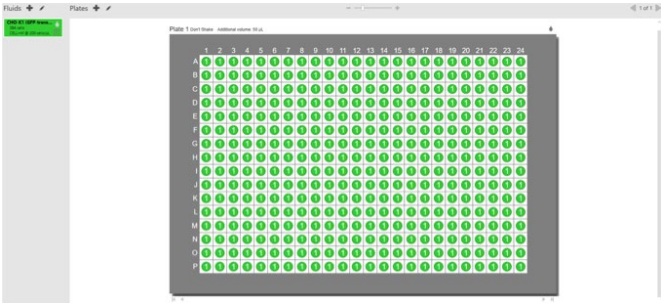

Figure 3: 384-well plate cell dispense protocol with the cell fluid applied to the plate.

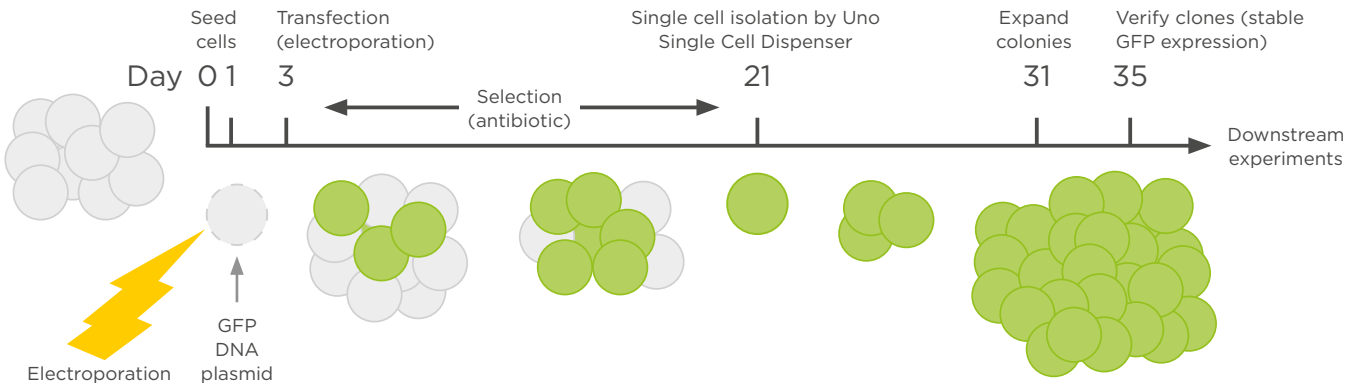

Figure 1: Timeline of single cell cloning experiment from transfection and selection to isolation and expansion.

Figure 4: Cell dispensing report with single cell isolation success rate results.

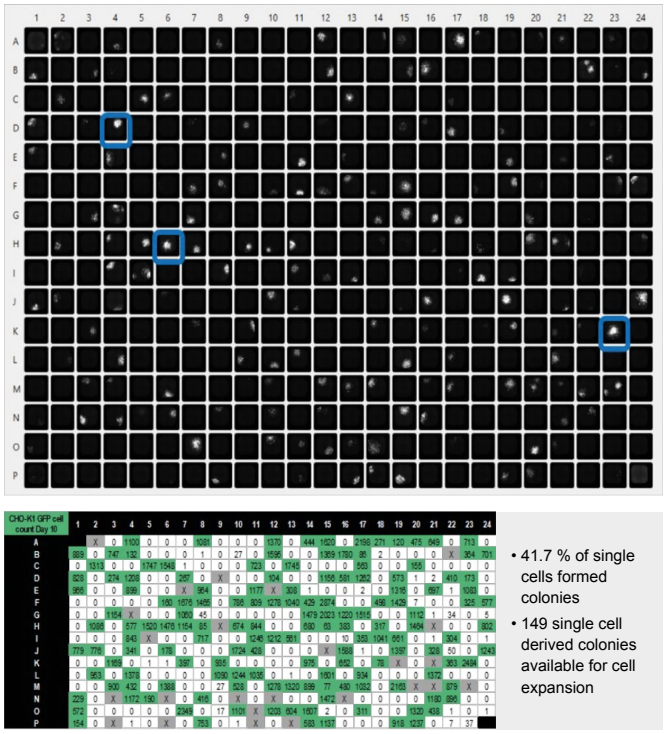

Figure 5: GFP fluorescence identifying a number of GFP-expressing single cells that formed colonies, with the brightest GFP-expressing clones selected for expansion (outlined in blue). Day 10 post single cell isolation (day 31 in Figure 1)

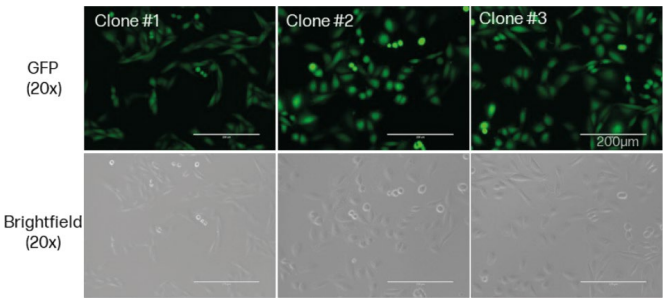

Figure 6: GFP-expressing clones expanded in large format dishes. Day 14 post single cell isolation (day 35 in Figure 1).

RESULTS.

Single cell isolation of CHO-K1 cells transfected with GFP plasmid and selected with antibiotics had a isolation success rate of 93 % across a 384-well plate (Figure 4). Following isolation, cells were expanded for 14 days, then scanned with fluorescent microscopy to look for the number of colonies formed. It was found that 41.7 % of single cells grew into GFP-expressing colonies (Figure 5). Three of the brightest GFP-expressing colonies (outlined in blue boxes in Figure 5) were further expanded into large-format culture dishes and monitored and confirmed for stable GFP expression for three passages and after a freeze thaw cycle (Figure 6).

CONCLUSION.

- CHO-K1 cells stably expressing GFP were generated using Uno Single Cell Dispenser for the cell isolation step.
- The Uno Single Cell Dispenser achieved a 93 % success rate in isolating transfected CHO-K1 cells across a 384-well plate.
- Utilizing the Uno Single Cell Dispenser for the isolation of GFP-expressing CHO-K1 cells reduced the cell isolation time to under 10 minutes.

Figure 7: The Uno Single Cell Dispenser is a compact, automated benchtop device engineered for simplicity. It gently and accurately dispenses single cells or reagents, delivering volumes from picoliters to microliters within minutes per 384 well plate.

ABOUT THE AUTHORS.

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